Analysis of three wetland medicinal plants: *Centella asiatica, Cyperus longus and Typha capensis* found in the Western Cape Province of South Africa.

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## An analysis of three wetland medicinal plants in Western Cape Province of South Africa

#### Keywords

Centella asiatica

- Cyperus longus
- Elemental analysis
- Macro elements
- Micro elements
- Phytochemical analysis
- Typha capensis

Seasons

- Western Cape
- Wetland medicinal plants



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#### Abstract

South Africa is recognised worldwide for its rich diversity of plants, many of which have been used in ethno-medicine. However, the use of wetland plant species in ethno-medicine required further investigations. This research is aimed at investigating three wetland medicinal, plant species, Centella asiatica, Cyperus longus and Typha capensis based on their geographical, seasonal, mineral nutrient (Fe, Mn, Ca, Mg, K and Na) and secondary metabolite characteristics. Samples of each species were collected from Grabouw, Kelderhoff, Kenilworth, Pringle Bay, University of the Western Cape (UWC) and Worcester within the Western Cape Province of South Africa. Specimen and soil collections were carried out during autumn, spring, summer and winter of 2014. Both plant and soil samples were acid digested and mineral nutrient concentrations in the samples were analysed using an atomic absorption spectrometer (AAS). Secondary metabolites were determined using analytical TLC on normal phase Merck-Silva gel coated aluminium plates as well as by using HPLC separation from crude extracts of C. asiatica, C. longus and T. capensis using LC-MS hardware from Agilent. The elemental analysis of soil samples showed that Ca, K, Mg, Mn and Zn concentrations were predominantly low. Soil mineral concentrations increased progressively from inland (Worcester) towards the coastland in the south (Pringle Bay). Calcium and sodium concentrations, in particular, were higher in soil samples obtained from Grabouw (inland south) and decreased northward towards Worcester. Comparatively, plant mineral concentrations were generally higher than soil concentrations. The high concentration of some of these essential elements, in selected plants is an indication that these plant species could be a good source of essential elements. High concentrations of phytochemicals were found in *Centella asiatica* during winter, while *Cyperus longus* and *Typha capensis* exhibited high concentrations during autumn indicating variation in respect of season. Consequently, harvesting of the studied plants should be done at the season with a relatively high phytochemical concentration. Studies are needed to investigate the extent of pesticide or herbicide contamination in wetland plants to protect the health of users.

The LC-MS analyses of the three study species showed that seasonal variation affects metabolite constituents and moreover that these metabolite constituents differ from one locality to another. The seasonal variation of the elements in the studied medicinal plants justified the importance of harvesting seasons in the optimal utilization of the studied plants for medicinal purpose. s, for *C. asiatica*, anti-bacterial treatments for *C. longus* and fertility enhancement and birth control for *T. capensis*.

#### Declaration

I declare that "An analysis of three wetland plants *Centella asiatica*, *Cyperus longus* and *Typha capensis* found in the Western Cape Province of South Africa" is my own work, that it has not been submitted before for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged as complete references.

Olusola Surajudeen Saibu



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## List of abbreviations

AAS	Atomic Absorption Spectrometry
A-MQ-TOF	Accurate Mass Quadruple Time of Flight
ANOVA	Analysis of Variance
Ca	Calcium
ESI	Electrospray Ionizer
Fe	Iron
GC-MS	Gas chromatogram mass spectrometry
mg/kg	Milligram per Kilogram
mg/L	Milligram per Litre
µS/cm	Micro-second per centimetre
Κ	Potassium
LC-MS	Liquid chromatogram mass spectrometry
Mg	Magnesium
Mn	Manganese
Na	Sodium
TLC	Thin Layer chromatography
UK	United Kingdom
UWC	University of the Western Cape
Zn	Zinc UNIVERSITY of the WESTERN CAPE

#### **1. CHAPTER ONE: INTRODUCTION**

Indigenous medicines can be defined as a combination of plant and/or animal products used for treatment, prevention and diagnostic purposes of ailments (Petrovska, 2012). Medicinal plants, in particular, have been used for centuries to treat different categories of disease ranging from diabetes to common typhoid fever (Isely, 2002; Magner, 1992; Petrovska, 2012; Saleh, 2011; Sumner, 2000; Wellisch, 1978). According to the World Health Organization (WHO, 2011), medicinal plant practice is "the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness" (Sato, 2012). The use of medicinal plants in healthcare is considered to be economically, cognitively and pharmacologically effective (Lai, 2015; Leonti, 2013; Savithramma, et al., 2011). The commonly held belief is that the exorbitant cost of modern healthcare and inaccessibility to these services has led to the predominance of indigenous medicine in rural areas (Liu, 2011). Also, it could be argued that is has more to do with tradition and lack of modern medical services in these areas (Willow & Liu., 2011; Yuan Haidan, et al., 2016). However, the literature shows that it is extensively applied in urban areas as well, most likely as a result of reported efficacy in treatment and cost effectiveness (Cunningham, 1988; Van Wyk, et al., 2009; Wangchuk & Tobgay, 2015). This would appear to be true on both a local as well as global scale (Abbasi, et al., 2009).

#### 1.1. Medicinal plants in a global context ERSITY of the

Recently, the medical uses of plants have gained renewed attention. More than 75% of the population in the continents of Africa, Europe and North America are using them as their first line of treatment for life-threatening diseases such as acquired immune deficiency syndrome (AIDS) and malaria (Ameh, *et al.*, 2010; Anquez-Traxler, 2011). Likewise, in Asian and South American countries, especially those with densely populated rural communities, such as India and China, depend extensively on natural medicinal products for their primary health needs (Ameh, *et al.*, 2010). Using traditional systems of healthcare is a global practice and almost 80% of the world's population depend on it as a form of treatment (WHO, 2011). Moreover, the general perception that formal education significantly reduces the preference for/application of indigenous healthcare may not be absolute, as studies in the recent past have found that socioeconomic factors such as age, education, gender and wealth do not determine individual preferences with regards to type of medication (Botha, *et al.*, 2004; Dold & Cocks, 2002).

Ahmad (2002) and Awodele, *et al.* (2013) showed evidences to suggest that the rise in popularity of natural medicines is linked to firstly, the increasing awareness of potential drug-resistance and the documented organ toxicity associated with synthetic pharmaceuticals and secondly, capital intensive

drug-development, which often leads to socio-cultural discrimination in access to synthetic pharmaceuticals.

Medicinal plants contain bioactive phytochemicals that play a major role in the prevention and/or treatment of chronic diseases such as cancer (Jung, 2014; Thomas, et al., 2015), diabetes (Afolayan & Sunmonu, 2010; Baharvand-Ahmadi, et al., 2016) and coronary heart disease (Pragada, et al., 2004). Plant-derived products are increasingly accepted and used in important cosmetic industries (Ivanova, et al., 2005). Large varieties of plants are reported to contain a broad range of bioactive secondary metabolites such as flavonoids, alkaloids, steroids, glycosides and terpenoids (Palombo, 2006; Pradeep, et al., 2014; Wink, 2010; Wulandari, et al., 2016). Showkat, et al. (2013) demonstrated that these compounds are active in their crude and refined forms. Pharmacological classes of drugs include natural product prototypes such as aspirin, atropine, ephedrine, digoxin, morphine, quinine, reserpine and tubocurarine, to name a few (Russo & Garbarino, 2008). The aforementioned examples are all substances originally discovered through intensive research in natural medicine and based on the knowledge of indigenous peoples (Russo & Garbarino, 2008), yet, the opportunity for bioprospecting of plant compounds for novel pharmaceuticals remains largely untapped (Street & Prinsloo, 2012). The claim here is that the recognition of the knowledge of traditional uses of medicinal plants is a vital step in modern pharmacological studies and treatment of diseases. The world herbal trade currently stands at approximately US\$ 120 billion with the possibility of reaching a potential US\$ 7 trillion by 2050 (Birada, 2015). In essence, medicinal plants are source of nonnarcotic drugs that have few side-effects, are easily available at affordable prices and sometimes the only source of healthcare available to the poor. Consequently, to develop the health sector for effectiveness and safety, especially in low-income countries, there is a moral and social-economic imperative for intensive research in natural medicinal products and raises the question of logistical and ethical issues around the availability, collection and sustainable harvest of medicinal plants (Ogundaini, 2005).

Several habitats enable easy propagation of plants with medicinal quality and usage, but the majority of the medicinal plants used in the herbal trade are gathered from the wild (uncultivated) (Cunningham Anthony, 2014). The collection of medicinal plants from the wild is an important source of livelihood, with local and foreign buyers, for many in the developing countries (Alan C.Hamiltona, *et al.*, 2016). Useful medicinal plants can be collected from the wild as well as cultivated (Hishe, *et al.*, 2016). The type of plant found in a habitat depends on the climatic conditions and other factors such as annual rainfall, type of soil and other abiotic factors. For example, secondary forests are well endowed with plants containing compounds useful in medicine compared to primary forest (Schippmann, *et al.*, 2006).

Another important habitat and source of many medicinal plants is wetlands (Horwitz & Finlayson, 2011). Wetland habitats of the Mediterranean, Central Asia, South East Asia and Northern Africa, in particular, have a significant number of medicinal plants (Cunningham, 2015; Horwitz & Finlayson, 2011; Kumar, *et al.*, 2011; Leaman, 2016). Wetlands are a diverse range of water based environments upon which human anthropogenic activity is very high (Bird & Day, 2014). They are shallow coastal environments such as estuaries and mangrove systems (Horwitz & Finlayson, 2011). Also, categorized as wetlands lakes, rivers, streams and marshes (Derne, *et al.*, 2015; Cunningham, 2015; (EPA.gov., 2017). Wetland medicinal plants, which include sedges (Cyperaceae), mint (Lamiaceae), knot weed (Polygonaceae), daisy (Asteraceae), ginger (Zingiberaceae), grass (Poaceae), and Arum family (Araceae), are very useful for their therapeutic values (Horwitz & Finlayson, 2011), containing significantly high fractions of trace elements found in different parts of the plants (Kartnig, 1988; Majid, *et al.*, 1995).

In-spite of their documented constitution of medicinally useful plant species, there is little attention paid to wetland medical plants compared to the plants from other habitats (Herbst, 2015). Given the widespread and intense degradation global wetlands suffer, this inattention is problematic. According to Herbst (2015), The Economics of Ecosystems and Biodiversity (TEEB), an estimated 50% decline in wetlands was reported in the last century and only an estimated 12.8 million square kilometres of wetlands remain in the world (Russi, *et al.*, 2013). A consequence of this is the loss of the world's most significant contributor to people's survival and sustainable development (Duraiappah, *et al.*, 2005). The value of wetlands to the health of people in low income countries, such as South Africa, cannot be overemphasised (Duraiappah, *et al.*, 2005). Despite this, wetlands remain the most threatened ecosystems in South Africa (Herbst, 2015).

South African wetlands comprise 275 plant communities as reported in a survey of wetland vegetation distribution and conservation importance (Sieben, *et al.*, 2014). These are classified into eight main vegetation types: aquatic submerged, montane grassy, saline and brackish, sclerophyllous (restricted to a specific region), short-term grassy, subtropical, swamp forests and temperate grassy wetland vegetation. The four latter types are mostly widespread (Sieben, *et al.*, 2014). Wetland communities in the Western Cape Lowlands are ranked highest in South Africa as they have the most species, contrasting with the montane regions wetlands of the Eastern Cape and KwaZulu-Natal (Sieben, *et al.*, 2014). Ramjukadh (2014) reported that in Western Cape of South Africa, there is a significant difference between several historical and present wetland communities. Moreover, the change in plant communities does not appear to be driven by the environmental variables, but rather by changes in the surrounding land-use.

#### 1.2. Medicinal plants in South Africa

South Africa has a remarkable biodiversity with more than 30 000 species of flowering plants (accounting for 10% of the world's higher plants), more than 10% of which are medicinally active (Van Wyk, et al., 2000). The South African vegetation is recognised around the world for its extensive diversity of plants species and many of these plants have been used since ancient times in ethnomedicine (Kishore & Lall, 2014). The world-renowned medicinally active South African plants include Cape aloes (Aloe ferox), "buchu" (Agathosma betulina) and devil's claw (Harpagophytum procumbens) (Hutchings, et al., 1996). Despite the remarkably large variety of medically active plant species in South Africa, there are few drug leads from the region. This may be due to lack of rigorous investigation on the transformation of the locally documented active herbal products into modern pharmaceuticals. However, the compounds that have been discovered are those of the appetite suppressant P57 isolated from Hoodia gordonii (Van Heerden, et al., 2007; Van Wyk & Wink, 2004) and anticancer compound combretastatin, isolated from Combretum caffrum (Griggs, et al., 2001). Furthermore, the ethnobotanical survey of plants used for the cure of gastrointestinal disorders in the Eastern Cape province of South Africa revealed that the infusion, decoction and concoction of the leaves and roots of *Centella asiatica* (L.) Urban and *Typha capensis* (Rohrb.) N.E. Br is good therapy for diarrhoea, stomach disorder and dysentery (Olajuyigbe & Afolayan, 2012). Further studies on these types of products, especially those that are found in wetlands regions are essential for bridging the knowledge gap in the ethnobotany and traditional healthcare industry of this region and the world **UNIVERSITY** of the at large.

South African vegetation is known for its strong climatic gradient (Goldblast & Manning, 2002). There are dry climates in the west, where saline and brackish wetland vegetation and short-lawn grassy wetlands are dominant, whereas, the eastern region has mesic climates, where temperate grassy wetland and subtropical wetland vegetation are most prominent. It is well known that in a single wetland, communities can comprise various types of plants (Sieben, *et al.*, 2014). Consequently, the varied climatic conditions have significant influence on the medicinal value of the plants, particularly since these regions are ideal for the growth of most wetland plants.

#### 1.3. Wetland plants in traditional medicine

The terrestrial habitat is dominated by several medicinal plants such as *Hypoxis hemerocallidea* Fisch.Mey & Ave Lall, African potato, *Aloe ferox*, Mill, *Sutherlandia frutescens* (L). R. Br - Cancer bush, *Hoodia gordonii*, *Moringa oleifera* Lam. and *Tulbaghia violacea* Harv. (Alliaceae) are of high commercial value utilised by local communities for diverse healing purposes (Nzue, 2009). Most of these medicinal plant stocks are already depleted, or near depletion, because of increased anthropogenic activities (Shengji, *et al.*, 2009). Therefore, proper and appropriate conservation

management practices must be applied in order to safeguard the terrestrial habitat and encourage the regrowth of the overharvested medicinal plant species. Comparatively, the low levels of anthropogenic activity in aquatic and sub-terrestrial (underneath soil) ecosystems have left these ecosystems relatively intact. Interestingly, (Kumar & Narain, 2010) also provide some evidence for that sub-terrestrial habitats have the propensity to retain medicinally active compounds. Substantial populations of aquatic macrophytes contribute to the general proficiency and diversity of a healthy aquatic ecosystem (Flint & Madsen, 1995). Various aquatic floras have played fascinating roles in the lives of humans since primitive times, providing economic benefits such as paper pulp and fibre, as well as serving as a source of green manure for food production (Gupta, 1987). Currently, there is limited information regarding the medicinal properties of aquatic flora in South Africa. However, several reports exist on the aquatic flora of other countries such as India and Sri Lanka. The aquatic flora of Sri Lanka consists of 90 peninsular species, 7 non-peninsular species and 11 endemic species (Abeywickrama, 1995).

Generally, the elemental uptake by wetland plants differs among species, and is related, amongst other factors, to the depth of the plant root (Guilizzoni, 1991). Broad ranges of elemental composition were noted within the same type of wetland depending on the geographical location (Reddy & DeLaune, 2008). An investigation of elemental concentration in the roots and shoots of four perennial wetland species, *Myriophlum aquaticum* (Vell.) Verdc. *Nymphaea odorata* Aiton (deep wetlands), *Juncus effusus* L., and *Pontederia cordata* L. (shallow wetlands), has shown that there are significant differences in the elemental concentrations between shallow and deep wetland species (Collins, *et al.*, 2005). The deep wetland plant species contain a higher concentration of sodium (Na), manganese (Mn) and phosphorus (P), while the shallow wetland plants nevertheless, have higher concentrations of zinc (Zn), aluminium (Al) and iron (Fe) (Collins, *et al.*, 2005). The findings of (Collins, *et al.*, 2005) highlight how sensitive wetland plant species are to the variation in the normal physiochemical parameters and climatic conditions. Therefore, planting and harvesting of wetland plants must be well-planned, with special consideration of the variable climatic factors in order to exploit their medicinal values (Saharia & Sarma, 2011). Table 1.1 shows some wetland medicinal plant species with their traditional medicinal uses and pharmacological effects.

Family plant species	Parts used	Distribution	Traditional & medicinal uses	Active ingredient	Pharmacological effects	References
<i>Acorus calmus</i> Linn. Acoraceae	Rhizome	India, Tropical Asia, North Temperate Hemisphere	Laxative, oral and stomach analgesi, antibiotic, anthelmintic, effective for bronchial catarrh, chronic diarrhoea, dysentery, fever and tumours.	Essential oils contain monoterpenoids (e.g. Camhene, p-cymene, linalool) and Sesquiterpenoids (e.g. acorenone)	Anti-dementia, antimicrobial, anti- epileptic, insecticidal and anti- diabetic activities.	(Balakumbahan, et al., 2010; Baxter, 1960; Bhuvaneswari & Balasundaram, 2009; Chopra, et al., 1957; Grosvenor, et al., 1995; Li, et al., 2017; Rani, et al., 2003; Sarma & Saika, 2010; Usher, 1984; Van Wyk, et al., 2009)
Ageratum conyzoies L. Asteraceae	leaf, root, flower, seed	Africa, Asia, Kenya, South America, Tanzania.	Healing of mental and infectious diseases. Purgatives, antiseptic, clotting, piles, uterine disorder.	Mono and sesquiterpenes, caryopyllene, polyoxygenated, flavonoids	Chronic pain, osteoarthrotic, anticonvulsant	(Okunade, 2002; Sarma & Saika, 2010)
<i>Centella asiatica</i> Urban Apiaceae	Leaf, stem, root	Asia, Africa, India, north America, Australia, South America	Memory enhancement, antifungal (skin diseases), anti-aging, bronchitis, dysentery kidney diseases, urethritis, ulcer prevention.	Triterpenoid, saponins, asistcosides, asiatic acid, madecassoside, madecassic acid and sapogenins.	Wound healing, antifungal, anti- inflammatory, tranquiliser, anti- hypotensive, peptic ulcer treatment, neuroprotective	(Abdulla, et al., 2010; Azerad, 2016; Cheng & Koo, 2000; Chopra, et al., 1957; Hong, et al., 2005; Kan, 1986; Kartnig, et al., 1988; Kaur, et al., 2016; Maquart, et al., 1990; Ncube, et al., 2017; Shetty, et al., 2006; Van Wyk, et al., 2009; Zainol, et al., 2003)
<i>Cyperus rotundus</i> L. Cyperaceae	Rhizome, tubers, roots	South India, Africa, Southern and central Europe, southern Asia, Australia	Diarrhoea, dysentery, analgesic, antibacterial, antispasmodic, antitussive, astringent, diaphoretic, diuretic, sedative, stomach tonic, anthelmintic, anti- malaria.	Alkaloids, flavonoids, tannins, glycosides, sesquiterpenes, sitosterol, glycerol.	Anti-candida, anti-inflammatory, antidibetic, anti-diarrhoea, cytoprotective, anti-mutagenic, anti-microbial, antioxidant, antipyretic, and analgesic.	(Al-Snafi, 2016; Cook, 1996; Parekh & Chanda 2006; Ranjani & Jeyaddevan, 2012; Shamkuwar <i>, et al.</i> , 2012)
<i>Cyperus longus</i> L. Cyperaceae	Leaf and root	Mediterreanean, Western and Central Europe, Tropical Africa, India, South Africa	Stomach aches,in children, diaphoresis	Flavonoids, alkaloids, cyperone, selinene, cyperusol A, cyperusol B <sub>2</sub> , C, D, menthene,-7, 8-diol, sobretol, cyperusol, sesquiterpenes, longsuone- A, B and C	Anti-bacterial.	(Memariani, <i>et al.</i> , 2016; Watt & Breyer-Brandwijk, 1962; Xu, <i>et al.</i> , 2004)
Typha capensis	Rhizome	Africa, South China, Japan, Germany, Turkey	Dysentery, diarrhoea, libido, dysmenorrhoea, veneral, diseases, and child birth.	Flavoniods, tyhaphthalid, typharin, sitosterol and epizelichin, (+) catechin and (-) epicatechin, Typarin, Typhasterol and Typhaphtalide	Fertility enhancement and birth control.	(Abdillahi & Van Staden, 2012; Hutchings, <i>et al.</i> , 1996; Masoko, <i>et al.</i> , 2008; Shode, <i>et al.</i> , 2002; Van Wyk, <i>et al.</i> , 2009; Watt & Breyer-Brandwijk, 1962)

#### Table 1.1: Characteristics of important wetlands medicinal plant species.

#### 1.4. Description of the three wetland medicinal plants in this study

The variety of medicinal plants in wetlands, known to traditional medical practitioners, that have yet to be rigorously studied to assess their pharmacological value, is extensive. The chemical properties of these medical plants are not well understood. The current increase in the development of natural medicine for the treatment of human diseases has led to increased awareness of the importance of medicinal wetland plant species (Kumar & Narain, 2010). Among these are freshwater plants that are being explored for symptomatic relief of various ailments. The widely used examples are *Zizania* spp. (wild rice), *Trapa natans* L. (water caltrop), and *Eleocharis dulcis* (Burm.f.) Trin ex Hensch, (Chinese water chestnut), *Nelumbo nucifera* Gaertn. (Indian lotus), *Ipomoea aquatica* Forssk. (water spinach), *Nasturtium officinale* – W. T. Aiton (watercress), *Neptunia oleracea* Lour. (water mimosa), *Colocasia esculenta* (L.) Schott. (Wild taro) and *Typha* spp. (cattails) (Ryan & John, 2012).

The City of Cape Town is one of the most developed cities in South Africa and the high rate of urbanization here has been linked to the loss of vegetation, wetlands and their invaluable medicinal plants (Sandberg *et al.*2014). The habitat loss and ecosystem degradation caused by urbanization in a city such as Cape Town cannot be overemphasised. It is important to investigate the influence of the climatic factors, which have undoubtedly been affected by urbanization, on the elemental composition of wetland plants in the Cape Town city area. This study focuses on the wetland medicinal plants in Western Cape Province (Cape Town); *Centella asiatica, Cyperus longus* L. and *Typha capensis*, were selected as study species because of their abundance and documented medicinal activities (Table 1.2.).

Medicinal	Plant	Country	Traditional and	Mineral	References
plants	part		medicinal uses	elemental	
	used			content	
Centella	Whole	Tropical Asia,	Nutritionally for	Fe, Mn,	(Abdulla, et al.,
asiatica	plant	Africa, India,	enhancement of good	Zn	2010; Chopra, et
		Madagascar	memory. Effective for		al., 1986; Cox, et
			the symptomatic		al., 1993; Hong,
			treatment of various skin		et al., 2005; Kan,
			diseases mostly wound		1986; Kartnig, <i>et</i>
			healing, leprosy. Also,		al., 1988;
			good for the treatment		Maquart, et al.,
			of bronchitis, dysentery,		1990; Shetty, et
			kidney trouble, urethritis		al., 2006).
			and protection against		
		5	ulcer.		
Cyperus	Tuber,	Europe, South	Effective in treatment of	Na, Ca,	(Ait-Quazzou, et
longus	whole	Africa	stomach aches in	Fe, Zn, K,	al., 2012; Watt &
	plant	4	children. For treatment	Mg, Mn	Breyer-
		U	of colds. The plant is		Brandwijk, 1962)
		W	therapeutic for <b>CAPE</b>		
			diaphoresis.		
Typha	Rhizo	South Africa,	Treatment of dysentery,	Ca, Mg,	(Hutchings, et al.,
capensis	me	Chinese, Japan,	diarrhoea,	Na, K,	1996; Watt &
		German,	dysmenorrhoea,	Zn, Fe	Breyer-
		Turkish	venereal diseases,		Brandwijk, 1962)
			enhancement of male		
			potency and libido, easy		
			delivery during		
			pregnancy.		

 Table 1.2: Useful mineral elements in medicinal plants studied in this thesis

#### 1.4.1.Centella asiatica (Pennywort)

Centella asiatica, a member of the Apiaceae, is a perennial creeper found throughout most tropical and subtropical countries (Ncube, et al., 2017; Saoji, et al., 2016) (Figure 1 A & B). Traditionally called Waternavel or Varkoortjies by Afrikaans speakers, this plant is used for the treatment of anaemia, bronchitis, cholera, constipation, diarrhoea, dysentery, epilepsy, hypertension, jaundice, leucorrhoea, nervous disorders, neuroprotection, anxiety, memory enhancement, smallpox, for a wide range of skin infectious diseases and as a cosmetic ((HMPC), 2010). Extracts of C. asiatica are applied to wounds for healing and poultices are used to treat closed fractures and sprains (Cheng & Koo, 2000; Saoji, et al., 2016; Zainol, et al., 2003). (Long, et al., 2012) reported that South African traditional medicine recognises two species of Centella, C. asiatica and C. glabrata L. Preliminary phytochemical screening has shown that amongst 16 species of the genus Centella, asiaticoside and madecassoside are the main medicinally active chemical constituents (Long, et al., 2012). The asiaticoside is an acceptable marker compound for quality control purposes. The plant has been extensively investigated both experimentally and clinically and has been proven to be an effective therapy for striae gravidarum, venous and healing of wounds (Brinkhaus, et al., 2000; Montecchio, et al., 1991; Oyedeji & Afolayan, 2005). Unlike other medicinal plants used for dermatological diseases, numerous scientific works have shown that C. asiatica contains active compounds such as saponins, sterols, flavonoids and tannins for treatment of dermatoheliosis, adiposis edematosa, stretch marks and inhibition of keloids, swollen stage scars as a result proliferation of fibroblast production, or the increased production of collagen and intracellular fibronectin (Bylka, et al., 2013). The successful use of C. asiatica as an indigenous therapeutic is as a result of the broad range of pharmacological activities due to the secondary metabolites known as centelloids (made up of pentacyclic, and triterpenoid saponins) and terpenoids (which consist of asiaticoside, centelloside, madecassoside, branmoside, thankuside, sceffoleside, centellose, asiatic-, brahmic-, centellic- and madecassic acids) (James & Dubery, 2009; Lokanathan, et al., 2016). The triterpernoids have antimicrobial properties that can be used for defense against pathogenic infections. Centella tritepernoids can be regarded as phytoanticipins (James & Dubery, 2009). There is future commercial prospective use for South African genotypes of *C. asiatica* compared to other global sources (Long, *et al.*, 2012).

#### 1.4.2. Cyperus longus

The Cyperaceae is the third-largest family of monocotyledons, one of the ten largest families of angiosperms (García-Madrid, *et al.*, 2015), (Figure 1 C & D). *Cyperus longus* has reportedly been used to cure some stomach disorders and colds (Watt & Breyer-Brandwijk, 1962). It is noted to be very toxic and injurious to the skin (Watt & Breyer-Brandwijk, 1962). However, a recent report explains that the essential oil present in *Cyperus longus* is an excellent antimicrobial due to the

presence of sesquiterpene (Ait-Quazzou, *et al.*, 2012). In a study of ecophysiological constraints of two invasive plant species under a saline gradient, looking particularly at halophytes compared to glycophytes, it was demonstrated that *Cyperus longus* a freshwater plant proliferating all around the Mediterranean was found to be negatively influenced by increased salt levels (Duarte, *et al.*, 2015). Furthermore, *C. longus* lacks the osmoregulatory ability to regulate itself in response to an ionic increase of the immediate environment; therefore, it colonizes the marshy area during the rainy seasons, which qualifies it as an opportunistic invader (Duarte, *et al.*, 2015). Memariani, *et al.* (2016) report that essential oils from *C. longus* have effects on PC 3 and MCF 7 cancer cell lines. Gas mass spectrometry (GC-MS) identified the 32 essential oils as aristolone,  $\beta$ - himachalene,  $\alpha$ - caryophyllene oxide,  $\beta$ - caryophyllene, irisone, longiverbenone, viridiflorol and humulene oxide (Memariani, *et al.*, 2016).

#### 1.4.3. Typha capensis

The family Typhaceae comprises numerous species, and amongst these are *Typha angustifolia*, *T. latifolia*, *T. minima*, *T. orientalis*, *T. domingensis*, *T. laxmannii*, among others (Londonkar, *et al.*, 2013). *Typha capensis* is a monoecious, robust perennial, a marshy and reed-like herb commonly called bulrush (Van Wyk, *et al.*, 2009), (Figure 1E & F). The height range of *T.capensis* is 3 to 4 metres it has stem, with long strap-shaped, hairless leaves about 10 – 20 mm broad (Van Wyk & Gericke, 2000). *Typha capensis* bears cylindrical inflorescences, which are tightly-packed (carpels) flowers, yellow initially and then turning dark brown thereafter when in fruit, beneath the (stamen) male flower. The stamen falls off, leaving the bulrush 'flower', which is in fact the fruit of the herb (Van Wyk, *et al.*, 2009). The fruit houses a small, hairy seed designed for wind dispersal (Van Wyk, *et al.*, 2009). The Typhaceae family is primarily distributed in the temperate and tropical areas of the world (Van Wyk & Wink, 2004).

*Typha capensis* is widely distributed in South Africa, though it is not endangered as is commonly believed (Van Wyk & Wink, 2004). Members of this plant family have multiple uses, such as food for humans, feed for animals and medicines to 'cure' various ailments. Traditionally, *T. capensis* has been used as a treatment for male infertility in South Africa. Investigations into the effects of *T. capensis* on male reproductive functions indicate that extract of *T. capensis* enhances production of testosterone at low concentration of Quercetin and Naringenin and might be useful to treat infertility and aging male malfunctioning (Henkel, et al., 2012; Watt & Breyer-Brandwijk, 1962). Investigations into the effects of *T. capensis* on male reproductive functions indicate that *T. capensis* enhances the production of testosterone at low concentration of Quercetin and Naringenin and might be useful to treat infertility to treat infertility and aging male malfunctions. Researchers have reported *T. capensis* to contain

phenolic compounds known as typharin and typhaphthalide (Shode, *et al.*, 2002). Studies have also shown that plant samples of *T. capensis* collected in summer exhibited potent biological activity compared to other seasons (Ilfergane, 2016).

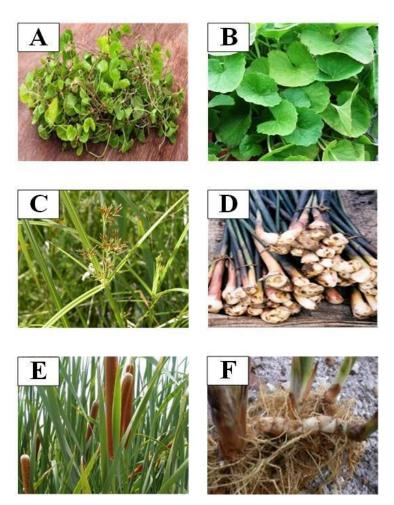


Figure 1.1: Three wetland plants *Centella asiatica* (A & B), *Cyperus longus* (C & D) and *Typha capensis* (E & F).

## 1.5. The elemental composition of medicinal plants

There has been extensive research conducted on organic constituents of medicinal plants such as alkaloids, amines, glycosides and tannins (Aires Alfredo, *et al.*, 2013). Relatively little is known about the inorganic constituents of bioactive plants and their contribution to the efficacy of these plants, medicinally. Quantification of the inorganic constituents in medicinal plants is highly desirable as these elements play important roles in the functional maintenance and regulation of cell organelle's (Reddy Byreddy, *et al.*, 2013). They are also responsible for enzyme production by acting as co-factors. The elements required by humans in large quantities are known as macronutrients, while micronutrients or trace elements are needed in small quantities, that is, less than 100 mg/day (Fraga,

2005). Macronutrients include calcium (Ca), chlorine (Cl), magnesium (Mg), potassium (K) and sodium (Na). Examples of micronutrient elements are chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), vanadium (V) and zinc (Zn) and non-metal trace elements are fluorine (F), iodine (I) and selenium (Se). Among the known mineral elements in living organisms, 23 have been reported to stimulate physiological activities in human, and 11 of these are classified as micronutrients (Street, 2012). The elemental concentration of medicinal plants is influenced greatly by the plants' genetic makeup, as well as environmental factors such as the nature of the soil and the annual rainfall (Bhowmik & Badal, 2012; Rehman, *et al.*, 2008). See Table 1.3 for a brief outline of the mineral contents and their functions in plants and animals.



## Table 1.3 Mineral contents and their functions in animals

Essential Elements	Functions in animals and plants
Calcium [Ca]	Important for bones and teeth formation, muscular system and heart functions and blood clotting, proper nerve functioning and improvement in the body immune defences (Bhowmik & Badal, 2012; Hamilton, <i>et al.</i> , 1988). Mostly present in the cell wall and vacuoles for secretory processes and cell wall stabilization.
Data asium [K]	
Potassium [K]	It functions in diuretic balance. It can also compliment Na for prevention of high blood pressure (Kolata, 1982). It may be responsible for transportation of metabolites in <i>Typha capensis</i> (Bhowmik & Badal, 2012).
Sodium [Na]	Prevention of hypertension, arteriosclerosis (Doyle, 2008; Logan, 2006). Sodium, Cl and K are electrolytes that maintain normal intravascular and extracellular
	fluid balance and a proper balance of acids and bases in the body. The function of sodium in plants is the regulation potassium and calcium intake (Maathuis,
	2014).
Magnesium [Mg]	Importance for plasma and extracellular fluid which helps for maintenance of osmotic equilibrium. It is needed for many enzyme-catalyzed reactions particularly
	with nucleotide that has magnesium salt (Rita, 2009). Mg <sup>2+</sup> act as central atom of the chlorophyll molecule, its fluctuations in the chloroplast regulate the
	enzymes during photosynthetic activity (Shaul, 2002).
Copper [Cu]	Copper is necessary for strong bones, hair and skin colour, haemoglobin formation in red blood cells, healthy protein and enzyme production (Razic, et al.,
	2003).Cu2+ is required for cell wall synthesis, to name only a few of its cellular tasks is important for photosynthesis and mitochondrial respiration, for carbon
	and nitrogen metabolism and for oxidative stress protection (Hänsch & Mendel, 2009).
Iron [Fe]	Essential for the formation of haemoglobin and also plays an important role in oxygen and ion transfer, with normal functioning of the central nervous system as
	well as the oxidation of food metabolites (Abbasi, et al., 2009; Mahapatra, et al., 2012). About 80% of the cellular iron is found in the chloroplast that is
	consistent with its major function in photosynthesis. It is involved in photosynthesis, mitochondrial respiration, nitrogen assimilation, hormone biosynthesis
	(ethylene, gibberellic acid, jasmonic acid), production and scavenging of reactive oxygen species, osmoprotection, and pathogen defense as redox-active metal
	(Hänsch & Mendel, 2009).
Manganese[Mn]	Aids in the reproductive functions and production of mother's milk (Razic, et al., 2003). Mn serves as a cofactor in essential processes such as photosynthesis,
	lipid biosynthesis and oxidative stress in plants (Socha & Guerinot, 2014).
Zinc [Zn]	Zinc played important role in the growth and development of children (Hussain, et al., 2009; Osendarp, et al., 2003; Sian, et al., 2002). It is a functional unit of
	many enzymes. It is present in the insulin and play active roles in the formation of genetic materials, proteins and development of the foetus (Hamilton, et al.,
	1988). Zn is an essential component of thousands of proteins in plants (Broadley, et al., 2007).

The parent rock from which soil is formed also determines the variation in the concentration of the macro and micronutrient elements available to plants (Rawlins, *et al.*, 2012). Soil pH also directly affects the bioavailability of many nutrients needed for proper growth and development of plants, most tending to do well in a soil pH range of 5.5 to 7.5. However, as pH decreases, so the quantity of nutrients in plants also decreases (Deng, *et al.*, 2004; Hanlon, 2009). The availability of a high concentration of beneficial element is the key for medicinal plants. There is high correlation between the elemental constituents of the medicinal plants and their phytochemical concentration (Zubek, *et al.*, 2015). The elements present in plants also play an important role in secondary metabolism and serve as foundation for phytochemicals such as alkaloids, flavonoids and phenolic compounds etc. (Mishra, *et al.*, 2012).

#### **1.6.** Phytochemical constituents of wetland plants

Phytochemicals are non-nutritive substances produced by plants primarily serving to protect the plant from herbivores or other invading organisms, which may cause detrimental effects to its normal functions. They also protect plant cells from environmental hazards such as droughts, pathogenic attacks, pollution, and excessive ultraviolet light, while, some of the phytochemicals contribute to plant flavours and colouration (Wink, 2003). Phytochemical compounds such as flavonoids, alkaloids, glycosides, phenolic compounds, terpenoids, organic acids, lipids and monosaccharaides are pharmacologically active. Recent publications have demonstrated the protective role of phytochemicals against organisms responsible for causing human disease (Wink, 2015). Consequently, they are well utilized in the research and development of new medicinal products (Jan, 2011).

#### **1.7.** Screening of phytochemical compounds

The most important step in the analysis of medicinal plants is extraction. This is necessary to obtain the desired phytochemical constituents for further characterization and separation (Sasidharan, *et al.*, 2011). Modern extraction techniques include solid phase micro-extraction, super critical-fluid extraction, pressurized liquid extraction, microwave-assisted extraction, solid phase extraction and surfactant mediated techniques (Sasidharan, *et al.*, 2011). The process of identification and characterization of bioactive compounds is also necessary, because plant extracts are commonly found as acombination of various types of bioactive compounds with different polarities (Sasidharan, *et al.*, 2011).

These bioactive compounds can be isolated by various isolation methods such as chromatography, or thin layer chromatography (TLC). The separation is dependent on their charge, size and shape of the

molecules. TLC provides a chromatographic fingerprint within a short time (Cieśla, et al., 2015) and is the most widely used of all the simple chromatographic methods in the analysis of mixtures (Lade, et al., 2014). This is largely adopted for the rapid and positive analysis of extracts or sample preparations (Kumar, et al., 2013). It is also suitable for monitoring the identity and purity of extracts or analysing combinations of substances and phytochemical preparations. The degree of difference in the chromatographic 'fingerprints' can be utilized as 'markers' in the standardization of all extracts, in particular solvent system separating compounds at specific Rf values that differ from other plant extracts. Amongst the advantages of TLC are short time analysis, low cost, specific derivatization on the same plate and multiple detection possibility (Cieśla, et al., 2015). Currently liquid chromatography mass spectrometry (LC-MS) is primarily used to profile natural products and fingerprinting for quantitative analyses and qualitative control purposes. LC-MS is universal, sensitive and provides structural information about the compounds analysed, such as molecular weight, molecular formulae and diagnostic fragments (Brusotti, et al., 2014). This technique involves online spectroscopic methods and simultaneously has a significant role in the phytochemical study and quality control of natural medicine treatments (Brusotti, et al., 2014; Ju-Hee & Young-Joo, 2014). However, several limitations of LC-MS have become apparent, such as its expense, incompatibility with non-volatile buffers, compound dependent responses and eluent modifiers (Brusotti, et al., 2014).

The fingerprinting method is predominantly used to emphasize the profile of a sample matrix, which is adequate to give indications of the source and preparation method. The profile of medicinal herbs depends on the quality of the crude herb material source (Patel, *et al.*, 2010). The studies of untargeted compounds have led to an emerging approach to determine the composition of medicinal plant products. This approach simultaneously analyses various compounds in medicinal plants. The use of (LC-MS), has more benefits over nuclear magnetic resonance (NMR) and gas chromatography mass spectrometry (GC-MS) for the determination of available metabolites, structures and quantity of plant metabolites in medicinal plants (Commisso, *et al.*, 2013; Steinmann & Ganzera, 2011).

At present, quality control of medicinal plants is carried out through chromatic fingerprinting. This is an analytical method and qualitative control accepted by the World Health Organization (Tistaert, et al., 2011; Zhang, 2000). Incorporating both targeted and untargeted methods in a holistic manner resulted in a better means of plant extract profiling (Liang, *et al.*, 2010; Wang, *et al.*, 2004; Wolfender, *et al.*, 2010).

Profiling is the total analysis by hyphenated techniques, such as liquid chromatography mass spectrometry (LC-MS), capillary electrophoresis mass spectrometry (CEMS) and gas chromatography mass spectrometry (GC-MS). A mass-spectrometer performs specific resolution and this highly

chromatographic detector, gives comprehensive sample profiles for an absolute number of components (Halket, *et al.*, 2005).

Chromatographic fingerprinting is recommended as technique for herbal plants and is a reliable strategy for the quality control of complex mixtures. Fingerprinting produces chromatographic electrophoretic spectroscopic patterns that can be compared to a sample of proven efficacy. The degree of similarity can be carried out by using computer aided similarity evaluation system (CASES) software on the occurrence of common peaks to obtain correlation coefficients (Steinmann & Ganzera, 2011). The fingerprinting can be classified into 'targeted analyses' and non-targeted analyses.' Targeted GC-MS and LC-MS methods can be used where a relatively small number of analytes are described in advance. This method achieves precision, and great accuracy especially where stable isotopes (i.e. internal standards) are available and where other signals from of all other components are ignored (Halket, *et al.*, 2005). Non-Targeted Analyses of GC-MS and LC-MS are carried-out where all chromatographic peaks can be characterized by their mass spectral patterns and identification of these patterns are performed in further samples (Halket, *et al.*, 2005).

#### 1.7.1. Alkaloids

# nenenenen,

Alkaloids, of plant origin, are basic, insoluble compounds of nitrogen that produce soluble salt when combined with an acid and are physiologically active in the plant and animal (Lai, 2015). It is a white crystalline substance, with a bitter taste. The classification of an alkaloid is based on the plant source. Most alkaloids names end in "ine", for example, atropine (from belladonna), quinine (from cinchona), ergotamine (from cocaine or ergot), morphine (fromopium), reserpine (from rauwolfia), vincristine (from vinca), and caffeine (from xanthine) (Atanasov, *et al.*, 2015; Wink, 2015). They are important secondary metabolites with numerous pharmacological activities such as anti-inflammatory and antioxidant properties (Okwu & Emenike, 2006). The medicinal use of alkaloids had been linked to their cytotoxicity properties, which predominantly play a preventive role in the plants by poisoning external invaders (Gurib-Fakim, 2006; Nobori, 1994).

#### 1.7.2. Flavonoids

Flavonoids are ubiquitous group of plant components present in almost every photosynthetic plant organ (Wink, 2015). Generally flavonoids are considered to contribute to resistance against pathogens (Kerstin, et al. 2016). The biological effects associated with flavonoids are anti-carcinogenic, anti-inflammatory, antioxidant and free radical scavenging abilities (Wulandari, *et al.*, 2016). The flavonoids possess wider pharmacological, particularly anti-cancer, characteristics (Ravishankar, *et al.*, 2013), disrupting the initiation, promotion and progression of cancer through modulation of

various enzymes receptors pathways for apoptosis (Sak, 2014), angiogenesis (Gatne & Addepalli, 2013), cellular proliferation, differentiation, inflammation and metastasis. The ATPase and phospholipase A2 effects on membrane permeability and inhibition of membrane bound enzymes are exhibited because of flavonoids (Raju, *et al.*, 2014). The presence of the hydroxyl groups on the  $\beta$ -ring along reactive oxygen species (ROS) enhances the antioxidant capability of flavones (Eldhose Binil, *et al.*, 2013).

#### 1.7.3. Saponins

The naturally-available, active glycosides manufactured by plants are saponins (Elekofehinti, 2015). Saponins protect the plant against parasitic infections; it also gives humans defence against pathogens while boosting the immune systems (Talreja, *et al.*, 2017). There are several health benefits of saponins, chief amongst which are cancer and heart diseases risk reduction, control of cholesterol levels, bone health, prevention of diabetic complication and obesity (Marrelli, *et al.*, 2016; Talreja, et al., 2017). Saponins are comprised of galactose, glucose, glucuronic acid, rhamnose, xylose and methylpentose that are glycosidically linked to sapogenin of a hydrophilic (water soluble) and hydrophobic (fat soluble) sugar parts (Elekofehinti, 2015). Bile salts and cholesterols bind together by saponins in the intestinal tract to inhibit reabsorption of the cholesterols into the blood (Talreja, *et al.*, 2017). *Centella asiatica* possesses active compound of saponins such as ursane (madecassoside and asitiacoside) (de Costa, *et al.*, 2016).

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The foaming nature of saponins is one of its characteristics as it contains both phenolic and nonphenolic compounds that contribute to it being classified as an antioxidant. It is also traditionally used as a detergent, fish poisoning agent, and pesticide, among other advantages attributed to human health (Bhumi & Savithramma, 2014; Eldhose Binil, *et al.*, 2013).

#### 1.7.4. Tannins

Tannins possess an astringent flavour and indiscriminately bind proteins as commonly plant secondary metabolites (Ashok & Upadhyaya, 2012). Tannins have antioxidant properties that are useful in the prevention and treatment of degenerative illnesses such as cardiovascular disease and/or cancer (Siqueira, et al., 2011). The two major tannins are condensed and hydrolysable; the hydrolysable tannins are made up of glucose (monosaccharide or polyol) combined with gallic acid polymers, while the condensed tannins have a combination of proanthocyanidins and flavonoids (Zhang, 2015).

#### 1.7.5. Terpenes

Terpenes are natural compounds present in numerous organisms. There are more than 55000 terpenoids compounds (Guimarães, *et al.*, 2014). A terpene is any of a large group of volatile unsaturated hydrocarbons found in the essential oils of plants, especially conifers and citrus trees (Chizzola, 2013). Numerous terpenoids are economically demanded because of their uses as fragrances and flavours in food and cosmetics industry (Saxena, *et al.*, 2013). Terpenes are classified according to their number of isoprene units, which are either hemitepenoids, monoterpenoids, sesquiterpenes, diterpenes, triterpenoids and tetraterpenoids (Amorati, *et al.*, 2013). Terpenes are important attracting pollinators to flowers with their scents, and serve as growth regulators of plants (Tumlinson, 2014). Furthermore, medicinal uses include such characteristics as anti-cancer, anti-ulcer, anti-malarial, anti-carcinogen, hepaticidal and anti-microbial activities (Guimarães, *et al.*, 2014).

#### **1.8.** Climatic factors and medicinal plants

Climatic factors are abiotic components of the environment that influence the nature of plants (Endara & Coley, 2011). Generally, factors such as rainfall and water, light, temperature, relative humidity, air and wind may influence the growth and development of living things in the environment. The greatest threat to the integrity of the environment and survival of living thing is presented by the change in climatic conditions (Tong, et al., 2014). Plants metabolites vary across geographical environments where they are greatly influenced by the climatic factors (Endara & Coley, 2011). Other factors that affect the quantity and quality of plant metabolites are the plants adaptation to the environment, genotypic sorting; and anthropogenic activities (Moore, et al., 2014), etc. Climate factors, in association with other factors, pose serious concern specifically to wetlands and their water resources (Gouvea, et al., 2012). The vulnerability of wetland systems to changes in quantity and quality of their water supply cannot be denied. This invariably affects the wetland ecosystem in terms of the variety of plants it can sustain as well as the metabolites composition of those (Wulandari, et al., 2016). Despite the existence of genetic control, gene expression, and genotypes, the total content and relative proportions of secondary metabolites in plants may vary over time and space (Gouvea, et al., 2012). The influence of climatic factors on the number of metabolites present in a given plant has been extensively invetsigated (Gouvea, et al., 2012). Harvesting the plant when these putative bioactive compounds are at their highest concentrations would provide consistency in their chemical profile, thus ensuring the quality and efficacy of derived medicinal products. For example, the highest concentration of the principal triterpenes (flavonoid compounds and chlorogenic acid) in Centella asiatica can only be found in summer due to the effect of climatic factors (Arora, et al., 2002). Consequently, a harvest outside that period will not have the expected constituent secondary metabolites and therefore result in low bioactivity.

#### 1.9. This study

The wetland plants *Typha capensis*, *Cyperus longus* and *Centella asiatica* have interesting 'medicinal' properties that have made them sought after by traditional herbalists. However, little is known about the chemical constituents that might provide medicines derived from them with the therapeutic benefits that their users believe exist. It is therefore necessary to investigate these plants scientifically in relation to their traditional use, and this study is undertaken across five sites in the Western Cape Province where these plants are found.

This study therefore strives to meet the following aims:

#### 1.9.1. Aim 1

Assaying the elemental concentrations of Na, Ca, Fe, K, Mg, Mn and Zn in *Typha capensis*, *Cyperus longus* and *Centella asiatica* and relating the variations in the concentrations to season, geographical location, soil mineral composition and a few other selected chemical properties. Chapter 2 focuses on these environmental determinants of the micro- and macro-elemental variations in the three wetland plant species.

The relevant research questions are:

i. What elements are present in these medicinal wetland plants, and what are their concentrations?

ii. How do these concentrations relate to the elemental composition of the soils in which the plants grow?

iii. What is the effect of season on the elemental concentrations measured in the plants?

iv. What is the effect of geographical locality on the elemental concentrations measured in the plants?

#### 1.9.2. Aim 2

Assaying the phytochemical composition of wetland plants using HPLC and LC-MS. The work towards this aim is presented in Chapter 3.

The relevant research questions:

i. What is the seasonal variation of the phytochemical profile of plants?

ii. What is the geographical variation of the phytochemical profile of the plants investigated?iii. Is there a correlation between the elemental composition in the soil and the plants with the

phytochemical profile of the wetland plants?

### 1.9.3. Aims 3-5

The intention of the last chapter, Chapter 4, is to discuss the findings that emerged from Aims 1 and 2 in relation to the benefits that these plants are believed to offer as therapeutic agents.

For Aim 3, to assess each of the claimed health benefits in relation to the chemical constituents detected and that are known to be able to produce the claimed health benefit.

Towards Aim 4, to provide guidelines for harvesting of the resource, i.e. during which seasons and from which localities can plants be obtained that will provide optimal health benefits?

Lastly, in Aim 5, to look at the national and international health guidelines underpinning the safe consumption of the chemical constituents detected in the plants, and offer recommendations for the resources' usage by local people.



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#### 2 CHAPTER TWO MATERIALS AND METHODS

#### 2.1. Introduction

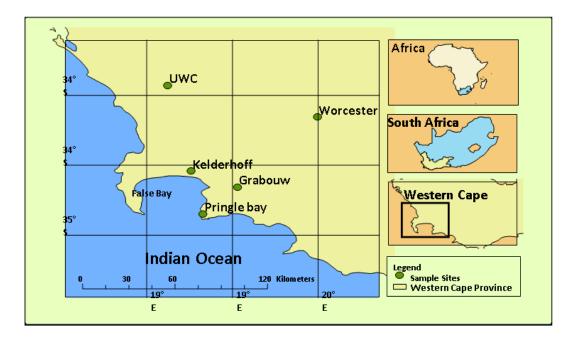
In light of the aims discussed at the end of Chapter 1, statistical significance tests focused only on those minerals that might be implicated as responsible for the health benefits that are reported to exist, for each species (see Table 1.2).

In regards to the secondary metabolites of the three species, samples were assayed qualitatively to provide an indication of the variation of these metabolites within samples for selected sites and seasons. Limitations in the sampling design preclude these samples from being subjected to hypothesis testing.

#### 2.2. Study areas

The sample locations were within a certain distance of Cape Town in South Africa (Table 2.1). These include Grabouw, Kelderhoff, Kenilworth, Pringle Bay, University of the Western Cape (UWC) and Worcester. The coastal wetlands are located at Kelderhoff and Pringle Bay while the inland wetlands are at Grabouw, UWC and Worcester (Fig 2.1).

Table 2.1: Geographical locations of sample collection areas					
Location	Latitude	Longitude	Altitude		
Grabouw	-34.158245	19.031681	324 m		
Kelderhoff	-34.041912	18.760357	24 m		
Pringle bay	-34.350404	18.829205	18 m		
UWC/Science	-33.431576	18.623766	61 m		
Worcester	-33.655602	19.498310	236 m		



#### Figure 2.1: The five selected sampling localities within a distance of the Western Cape

#### 2.3. Sample collection

Specimens of *C. longus* and *T. capensis* were collected randomly, (random sampling refers to a variety of selection techniques in which sample members are selected by chance, but with a known probability of selection) together with the representative soil samples from Grabouw, Kelderhoff, Pringle Bay, University of the Western Cape (UWC) and Worcester. For these two species, samples were collected during in each season throughout the year: winter (June-August), spring (September-November), summer (December-February) and autumn (March-May). Potted plant specimens of *C. asiatica* were obtained from the Stodels nursery in Kenilworth, during winter and summer, as they could not be sourced from their natural locations. The collected samples were stored in a plastic bag and transported to the Department of Biodiversity and Conservation Biology at the University of the Western Cape. Each stored sample was assigned an identification number (O. S. Saibu, *C. asiatica*, whole plant, 7065; *C. longus*, whole plant, 7064; *T. capensis*, rhizome, 7063) before depositing them at the University of the Western Cape Herbarium.

#### 2.4. Plant and soil mineral composition

#### 2.4.1. Sample preparation

The plant samples were carefully washed with tap water until there was no trace of contaminants such as organic matter. They were finally rinsed with de-ionised water and air dried for 24 hours. The dried samples were cut into smaller pieces for further drying and grinding. The dried, ground plant samples were stored in 200 mL polyethylene bags. They were labelled with brown paper bags and oven-dried

at 45°C until a constant mass was obtained. Similarly, the soil samples were air-dried in the laboratory within the pre-treated labelled plastic bags and kept open for free air circulation. The dried soil samples were sieved through a laboratory test sieve with an aperture of 1 mm. Both the air-dried and well-sieved soil samples were kept separately in plastic bags for further use.

#### 2.4.2. Soil pH

Dried and sieved soil samples 100 g were weighed into a 250 mL beaker, and a small quantity of deionised water was added while stirring. The soil mixture was stirred until it was wet enough to stick to a spatula, but damp enough not to puddle in a depression made in the surface. The mixture was allowed to stand for 10 minutes before an electrode was carefully inserted into pre-a prepared hole. The electrode was carefully covered with the damp soil for approximately 2 minutes for the electrode to make good contact to determine the pH level (Jackson, 1962) before it was recorded.

#### 2.4.3. Soil conductivity

The same damp soil sample used for sticky point pH measurement was used to fill a conductive cup to the brim. The filled conductivity cup was connected to a conductivity meter and read, the value obtained was multiplied with the cell constant  $(\mu S) \times (cm-1) = (\mu S \text{ cm}-1)$  (Jackson, 1962).

#### 2.4.4. Soil and plant digestion

Aqua-regia, digestion reagent used for the acid digestion analysis, was prepared overnight by mixing 300 mL HNO<sub>3</sub> with 900 ml HCl in a glass bottle and placed an ice bath housed in a fume cupboard (Chen & Ma, 2001). The soil and plant samples were digested separately, using the following steps: 0.5 g of the sieved soil sample was wrapped in cigarette paper (Rizzler), and was digested in Kjeldahl flasks in triplicate with 12 mL of the prepared Aqua regia digestion mixture. The flasks were covered with glass funnels to prevent the escape of HNO3 fumes. The digestion was done using a heating block at temperatures ranging between 100–380 °C in a closed fume chamber until a clear solution was obtained ( $\pm 6 - 8$  hours) (Chen & Ma, 2001). The residues of the Kjeldahl flasks were diluted with de-ionised water, and filtered through Whatman 42 filter paper. The final volume was made up to 100 mL using standard volumetric flasks and the digested sample was stored in polyethylene bottles for analysis.

The Atomic Absorption Spectrometry (AAS) analysis was done with the aid of a Unicam M-series Pye Solar Atomic Absorption Spectrophotometer (Unicam Unlimited, Cambridge, UK). With each digestion reagent, blanks were prepared for quality control purposes. 1000 mg.L<sup>-1</sup> stock solutions of each analyte (Na, K, Ca, Mg, Mn, Fe, and Zn) were prepared and the gradient made by diluting the blank with an appropriate amount of deionised water. The identification and quantification of the metals were determined in furnace mode.

#### 2.5. Statistical analysis of the plant and soil

All the data were analysed using the Statistical Package for Social Scientists (SPSS) version 23.2 for Windows (IBM) and R3.1.3 (Team, 2015) to compare differences in mineral concentration amongst locations. Statistical significance was declared at p < 0.05 in all analyses.

#### 2.6. Preparation of crude extracts

Dried, pulverised plant material (50 g each) was soaked in 80 % aqueous methanol and placed on a magnetic stirrer for 24 hours to homogenise the suspension. The process was repeated for four seasons (autumn, spring, summer, and winter) and five locations, (Grabouw, Kelderhoff, Pringle Bay, UWC Science, and Worcester), for two of the three study species, *C. longus* and *T. capensis. Centella asiatica* was collected for two seasons (summer and winter) from horticulture outfit located at, Kenilworth.

The methanolic extracts were filtered using Whatman no. 1 filter paper and the filtered extract was evaporated. Dried crude extracts of samples were preserved in labelled vials and allowed to evaporate to dryness in the fume chamber before being kept in a fridge until time of use. The preserved, refrigerated sample was collected with the tip of a spatula and dissolved in 1 mL of 80% methanol.

Analytical TLC was performed on both aluminium and plastic pre-coated plates of Merck Silica gel 60 PF254 with a 0.2 mm layer thickness. The TLC spots were sprayed with the p- anisaldehyde reagent prepared by mixing 0.5 ml anisaldehyde mixed with 10 ml glacial acetic acid, followed by 85 ml of chilled methanol and 5 ml of 98% sulphuric acid and visualised under UV light at 254 nm and 366 nm (Kotze, et al., 2002).

#### 2.6.1. Preparation of samples for LC-MS

Subsamples of 0.1g of each of *Typha capensis*, *Cyperus longus* and *Centella asiatica* the prepared crude extract samples kept in the fridge, were dissolved in 0.5 ml of methanol and filtered through a 0.45 micro-filter membrane for Liquid Chromatography Mass Spectrometry analysis.

#### 2.6.2. LC-MS analysis

For the chromatographic analysis, 20  $\mu$ l of each sample was injected into 0.3 ml/ min solvent flow. A flow of 100% solvent A was held for 3 minutes, followed by 18 minutes gradient to 40 % solvent B and the 5 minutes gradient till 100% solvent B. This flow was maintained for 5 minutes before being returned to 100 % solvent A over a three minutes gradient. The total gradient time was 18 minutes.

The solvent gradient or solvent used were: 97 % water, 3 % acetonitrile and 0.1 % formic acid (solvent A) with 90 % acetonitrile, 10 % water and 0.1 % formic acid (Solvent B).

## 2.7. Analysis of secondary metabolites

The software MZ-mine 2.0 was used for the LC-MS chromatograms results of *C. asiatica* (winter and summer) collected, from Kenilworth, *C. longus* (winter, spring, autumn and winter) collected from Grabouw, and Pringle Bay and *T. capensis* (spring, autumn and summer) collected from Grabouw and Pringle Bay. R-Studio was used for their analysis and for generation of bar and pie graphs.



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## 3. CHAPTER THREE: RESULTS OF ELEMENTAL AND PHYTOCHEMICAL ANALYSIS

#### 3.1. Elemental analysis

#### 3.1.1. Element concentrations in soils

The seasonal concentrations of the seven elements deemed important in this study were determined from the soil samples taken around the plants under investigation (*C. asiatica, C. longus* and *T. capensis*). Most of the soil samples do not show significant differences in the concentrations of the elements measured across the seasons (Figures 3.1 - 3.3).

## 3.1.1.1. Soil mineral concentration in *Centella asiatica*

The important minerals identified for *Centella asiatica* are Fe, Mn and Zn. All elements display some variation for the two studied seasons (winter and summer). *Centella asiatica* was sampled from only one locality (Kenilworth or Stodels) during two seasons (summer and winter) due its scarcity during the other seasons and in the studied province, Figure: 3.1.

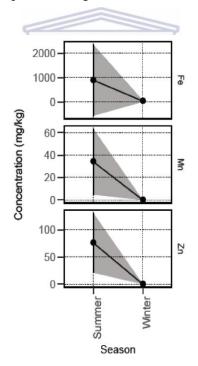


Figure 3.1: Concentrations of elements in the soil from Kenilworth for *C. asiatica* in summer and winter. Each point represents the mean and the bars represents plus or minus the SD

Figure 3.1 shows the concentrations of mineral elements in *C. asiatica* and soil samples obtained at different seasons (summer and winter) and three elements (Fe, Mn and Zn). There was no significant difference in the concentration between elements and seasons according to the Tukey post-hoc test, we find that p = 0.486. There was a small difference in the concentration of elements observed

between seasons in the soil. The lowest concentration observed in Mn and Zn was in winter, and the highest was in summer, generally, at Kenilworth, Fe was the highest during summer.

#### 3.1.1.2. Soil element concentration in *Cyperus longus*

The important minerals identified for *Cyperus longus* were Ca, Fe, K, Mg, Mn, Na and Zn. All elements display significant variation from season to season (winter, spring, summer and autumn) and from place to place (Grabouw, Kelderhoff, Pringle Bay, Science/UWC and Worcester) Figure 3.2. The concentration of Ca in the soil ranges from the lowest recorded value of  $1,570.65 \pm 827.58$  mg/kg at Pringle Bay during spring to the highest of  $10,415.24 \pm 437.00$  mg/kg during spring at Grabouw. These differences between seasons and localities were significant (2-way ANOVA: F = 12.65, d.f. = 3, p < 0.001 and F = 48.87, d.f. = 4, p < 0.001, respectively). Concentrations of Ca were generally highest during autumn, when it exceeds  $8,541.11 \pm 595.13$  mg/kg but the lowest concentrations were found during winter and spring.

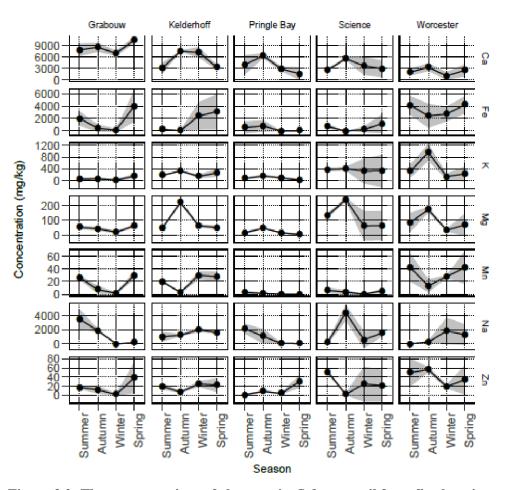


Figure 3.2: The concentrations of elements in *C. longus* soil from five locations across four seasons. Each point represents the mean and the bars represents plus or minus the SD.

The soil at Worcester contains the highest concentration of Fe ( $4220.62 \pm 713.24 \text{ mg/kg}$ ) during spring while the lowest concentration was noted at Pringle Bay ( $36.57 \pm 33.94 \text{ mg/kg}$ ) in the same season. Generally, concentration of Fe was highest during spring ( $4150.07 \pm 770.34 \text{ mg/kg}$ ) but lower during autumn and winter. There was a highly significant difference in Fe concentration between localities and seasons (2-way ANOVA: F = 12.88, d.f. = 4, p < 0.001 and F = 6.35, d.f. = 3, p < 0.01, respectively). The K concentration levels in the soil was at its lowest,  $11.19 \pm 6.75 \text{ mg/kg}$ , during winter at Grabouw and highest at Worcester during autumn ( $955.58 \pm 145.08 \text{ mg/kg}$ ). It was predominantly higher in autumn ( $398.79 \pm 32.81 \text{ mg/kg}$ ) with its lowest level in winter. There was also a highly significant difference in K concentration between localities and seasons (2-ways ANOVA: F = 10.07, d.f. = 4, p < 0.001 and F = 6.35, d.f. = 3, p < 0.001 respectively). The concentration ference in K concentration between localities and seasons (2-ways ANOVA: F = 10.07, d.f. = 4, p < 0.001 and F = 6.35, d.f. = 3, p < 0.001 respectively). The concentrations of Mg in the sediment differ from one location to another with the lowest value of  $3.87 \pm 3.87 \text{ mg/kg}$  recorded from Pringle Bay in spring. While the highest value was  $221.46 \pm 6.31 \text{ mg/kg}$  at Kelderhoff in autumn, and Mg concentration was generally high during autumn where it was more than  $221.46 \pm 6.52 \text{ mg/kg}$ . Magnesium was generally at its lowest in winter. These

differences between seasons and localities are significant (2-way ANOVA: F = 27.89, d.f. = 3, p < 0.0001 and F = 17.12, d.f. = 4, p < 0.0001, respectively).

The concentration level of Mn ranged from its lowest at  $1.41 \pm 0.72$  mg/kg in winter at Grabouw to its highest value of  $42.71 \pm 11.37$  mg/kg at Worcester during summer, but generally, it increases during spring and summer ( $29.87 \pm 3.20$  and 20.04 mg/kg) respectively. The seasonal and localities values were significantly different (2-way ANOVA: F = 13.274, d.f. = 3, p = 0.0001 and F = 31.60, d.f. = 4, p = 0.0001) in the soil. The concentration level of Na ranged from  $48.00 \pm 48.00$  mg/kg during spring in Pringle Bay to very high  $4402.03 \pm 570.01$  mg/kg during autumn at Science/UWC, further noted to be generally high in summer, recording levels above  $2165.77 \pm 360.20$  mg/kg at Pringle Bay. The locations and seasons were significantly different between (2-way ANOVA: F = 3.48, d.f. = 4, p < 0.05 and F = 5.03, d.f. = 3, p < 0.0001). The Zn concentration level range from  $1.27 \pm 0.87$  mg/kg in summer in Pringle Bay with high  $56.57 \pm 2.88$  mg/kg at Worcester in autumn, in addition, generally high  $24.58 \pm 19.54$  mg/kg in winter at UWC. The locations and seasons were significantly difference between (2-way ANOVA: F = 5.76, d.f. 4, p < 0.0001 and F = 3.21, d.f. = 3, p < 0.0001).

In summary, the concentration of elements in the soil was predominantly low in the following major elements: Ca, K, Mg and Na while the minor elements, Mn and Zn, were lower, with the exception of Fe. The concentrations of mineral elements at Grabouw were high during spring except Na. Kelderhoff has lower concentrations of mineral elements with the exception of Ca and Mg during autumn. Pringle Bay concentration levels of mineral elements were high during autumn, apart from Zn during spring as shown in (Figure: 3.2). The mineral concentration level was high at Science/UWC

during autumn, except for Fe and Zn. The concentration of mineral elements at Worcester was high for Fe, Mn and Na during autumn. Generally, the trend of mineral elements was high from Grabouw and decreased towards Worcester for Ca and Na, geographically, it can be observed that mineral concentration increases from in-land north (Worcester) southward to the coastland (Pringle Bay).

## 3.1.1.3. Soil element concentration in *Typha capensis*

*Typha capensis* contains the essential minerals Ca, Fe, K, Mg, Na and Zn. Most elements display some variations from season to season and from place to place (Figure 3.3). The Ca soil concentration ranges from the lowest recorded value of  $1,150.27 \pm 515.04$  mg/kg at Worcester during winter to the highest of  $10,591.76 \pm 599.24$  mg/kg during spring at Grabouw. These differences between seasons and localities were significant (2-way ANOVA: F = 6.791, d.f. = 3, p < 0.001 and F = 10.670, d.f. = 4, p < 0.001, respectively). Furthermore, concentrations of Ca were generally highest during autumn. It generally exceeds  $3,166.68 \pm 162.24$  mg/kg at lowest concentrations during autumn. The soil at Grabouw contains the lowest concentration of Fe 8.97  $\pm$  8.97 mg/kg during winter while the highest concentration was also at Grabouw 5197.91  $\pm$  2469.42 mg/kg in summer. Moreover, Fe was generally high during spring  $157.80 \pm 80.53$ . The level of significant difference between localities and seasons were (2-way ANOVA: F = 5.21, d.f. = 4, p < 0.01 and F = 4.738, d.f. = 3, p < 0.01, respectively).

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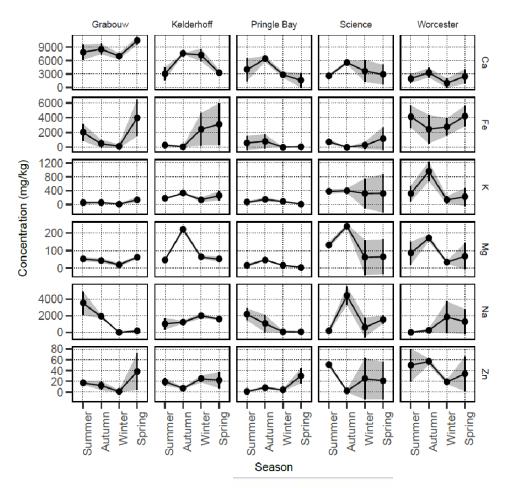


Figure 3.3: The concentrations levels of elements in *T. capensis* soil from five locations across all four seasons. Each point represents the mean and the bars represents plus or minus the SD.

The concentration of K ranges from  $62.87 \pm 33.76$  mg/kg during spring at Pringle Bay to very high  $5237.14 \pm 154.81$  mg/kg during winter at Kelderhoff, and generally high concentrations were found in autumn above  $397.14 \pm 149.84$  mg/kg at Worcester. The locations and seasons were significantly different between (2-way ANOVA: F = 41.54, d.f. = 4, p < 0.001 and F = 1.07, d.f. = 3, p < 1). The concentration level of Mg varied from  $0.97 \pm 0.89$  mg/kg during winter at UWC to very high  $417.10 \pm 17.02$  mg/kg during winter at Kelderhoff, further note to be extensively high in autumn above  $34.86 \pm 2.18$  mg/kg at Pringle Bay. The locality and seasonal were significantly difference between (2-way ANOVA: F = 82.734, d.f. = 4, p < 0.001 and F = 1.993, d.f. = 3, p < 1). The concentration of Na in *T. capensis* was lowest  $12.16 \pm 6.36$  mg/kg at Kelderhoff during autumn while the highest concentration was  $2896.21 \pm 227.98$  mg/kg in winter at Pringle Bay. The localities and seasons differences were significant between (2-way ANOVA: F = 2.450, d.f. = 4, p < 0.1 and F = 0.54, d.f. = 3, p < 1). The Zn concentration level was very high  $73.91 \pm 72.29$  mg/kg at Grabouw in winter and at extremely low values of  $19.66 \pm 10.09$  mg/kg; however, it was generally low  $1.28 \pm 1.04$  mg/kg in spring. The

locations and soils are significantly different between (2-way ANOVA: F = 1.674, d.f. = 4, p < 1 and F = 2.21, d.f. = 3, p < 1), (Figure: 3.3).

#### 3.1.2. Element concentrations in plants

The seasonal concentrations of the seven elements deemed important in this study were determined for the plants under investigation (*C. asiatica*, *C. longus* and *T. capensis*). Most of the soil samples did not show extensive variations in their elemental concentrations measured across the relevant seasons (Figure 3.4–3.6). K was the most abundant element in both *C. longus* and *T. capensis*, while the concentrations Ca and Zn were also relatively high.

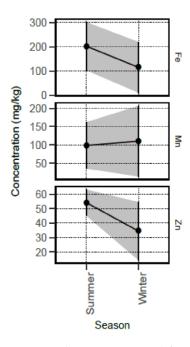


Figure 3.4: The concentration of elements (Fe, Mn and Zn) in *C. asiatica* from Kenilworth location in two seasons (winter and summer). Each point represents the mean and the bars represents plus or minus the SD.

## 3.1.2.1. Element concentration in *Centella asiatica*

Figure 3.4 shows the mean concentration of *C. asiatica* between summer and winter in three elements (Fe, Mn and Zn). There was no significant difference in the mean concentration between the three elements in the two seasons according to the two-way ANOVA test, where we find that p = 0.794. There was a small difference in the concentration of elements observed in the plant between seasons. The lowest average concentration observed in Zn was in winter ( $37.87 \pm 33.21 \text{ mg/kg}$ ) and the highest average concentration was in Fe during summer ( $202.61 \pm 101.18 \text{ mg/kg}$ ). At Stodels/ Kenilworth the average concentration of three elements was higher during summer than winter, Zn and Fe, while Mn had a slightly higher concentration in winter than it did in summer.

#### 3.1.2.2. Element concentration in *Cyperus longus*

The important minerals identified for *C. longus* were Ca, Fe, K, Mg, Mn, Na and Zn. All elements displayed significant variation from season to season (winter, spring, summer and autumn) and from place to place (Grabouw, Kelderhoff, Pringle Bay, UWC and Worcester) (Figure 3.5).

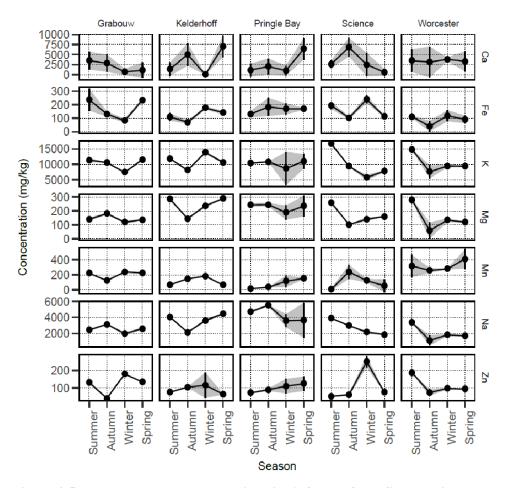


Figure 3.5: The elemental concentrations in *C. longus* from five locations across four seasons. Each point represents the mean and the bars represents plus or minus the SD

The highest concentration level of Ca, was recorded at UWC 6821.27  $\pm$  1190.21 mg/kg during autumn and the lowest concentration levels of 36.62  $\pm$  36.62 mg/kg were found at Kelderhoff in winter. The significant levels between locations and seasons were (2-way ANOVA: *F* = 1.18, d.f. = 4, *p* = 0.34 and *F* =5.13, d.f. = 3, *p* < 0.0001, respectively). Fe concentrations in *C. longus* were lowest 40.22  $\pm$  20.21 mg/kg in autumn at Worcester and the most concentrated Fe was found to be 238.13  $\pm$ 13.75 mg/kg at UWC during winter, with significant differences within localities and seasons (2-way ANOVA: *F* = 17.19, d.f. = 4, *p* < 0.0001 and *F* = 11.11, d.f. 3, *p* < 0.0001 respectively). As far as K concentration in *C. longus* was concerned, the findings revealed that it was highest compared to the amounts obtained in soil samples; however, the lowest concentration of 5768.01  $\pm$  435.14 mg/kg was 32

observed at Science/UWC in winter as well as the highest concentration  $16636.36 \pm 212.49$  mg/kg in summer. The significant level between seasons and locations (2-way ANOVA: F = 31.20, d.f. = 3, p < 0.0001, and F = 1.50, d.f. = 4, p < 0.0001). The concentration of Mg in *C. longus* was lowest 59.11  $\pm$  29.56 mg/kg at Worcester during autumn while the highest concentration 289.42  $\pm$  2.73 mg/kg was recorded in spring at Kelderhoff. The variation in elemental composition between localities and seasons were significantly different (2-way ANOVA: F = 48.96, d.f. = 4, p < 0.001 and F = 51.77, d.f. = 3, p < 0.0001). The Mn concentration was relatively high recording levels of 411.29  $\pm$  65.29 mg/kg at Worcester in spring and an extremely low value of  $6.29 \pm 6.29$  mg/kg, at UWC. However, winter concentrations of Mn were generally high, above 277.42  $\pm$  3.07 mg/kg. The Mn average concentration levels at the five locations as well as that of the soils were significantly different (2-way ANOVA: F = 46.66, d.f. = 4, p < 0.0001 and F = 4.76, d.f. = 3, p < 0.001).

Sodium concentration was high in autumn at Pringle Bay, but low at Worcester in the same season (Fig. 3.5). Sodium levels were generally high at  $3655.67 \pm 1112.20 \text{ mg/kg}$  in spring at Pringle Bay. The Na concentrations levels between localities and seasons were significantly different (2-way ANOVA: F = 43.98, d.f. = 4, p < 0.0001 and F = 13.56, d.f. = 3, p < 0.0001). The concentration of Zn was high,  $39.33 \pm 1.83$  mg/kg, in autumn at Grabouw increasing to  $187.84 \pm 1.41$  mg/kg in summer at Worcester and remained generally high during winter, recording levels of above  $115.31 \pm 37.12$  mg/kg at Kelderhoff. The locations and seasons were significantly different (2-way ANOVA: F = 4.521, d.f. = 4, p < 0.0001 and F = 38.56, d.f. = 3, p < 0.0001) (Figure: 3.5). Elemental concentration in *C. longus* was generally high compared to the soil samples, except for Fe. Calcium concentration was high at Kelderhoff, Pringle Bay and UWC during autumn and spring.

The concentration of Fe was generally low, but more abundant at Grabouw, Pringle Bay and Science/UWC during autumn, summer and winter, respectively. Potassium concentration was extremely high at Kelderhoff, Science/UWC and Worcester during summer and winter. The Mg concentration in the plant was relatively high compared with that of the soil; however, there was a high concentration level at Kelderhoff, Pringle Bay, UWC and Worcester during autumn and summer.

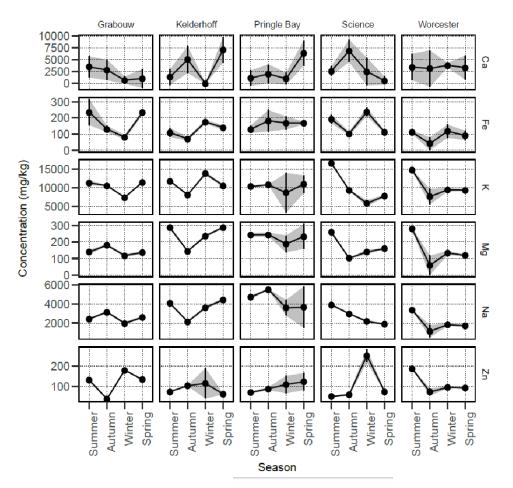


Figure 3.6: The concentrations of elements in *T. capensis* from five locations in four seasons. Each point represents the mean and the bars represents plus or minus the SD.

## 3.1.2.3. Element concentration in *Typha capensis*

Figure: 3.6 shows that the Ca concentration in *T. capensis* was highest with  $7246.22 \pm 1748.25$  mg/kg at Pringle Bay during spring, but in low concentrations at Science/UWC in winter. It is notable that while *T. capensis* from Grabouw has generally lower levels of Ca, during spring the concentrations were on par with other locations at above 2979.75  $\pm$  958.25 mg/kg. The sites and seasons were significantly different in their means (2-way ANOVA: *F* = 8.76, d.f = 4, *p* < 0.0001 and *F* = 2.29, d.f = 3, *p* < 0.0001).

Iron concentrations in *T. capensis* were at the highest level of  $268.17 \pm 13.22$  mg/kg at Grabouw during spring and at its lowest concentration level of  $39.30 \pm 18.60$  mg/kg in autumn at Worcester. The localities and seasons were significantly different in their means (2-way ANOVA: *F* = 20.79, d.f. = 4, *p* < 0.0001 and *F* = 2.19, d.f. = 3, *p* < 0.0001). Comparatively, Potassium concentration was significantly higher at Kelderhoff during winter with the lowest level of concentrations during autumn

at Worcester although the concentration level was mostly high at Worcester during summer (Figure 2.7). The mean concentrations of both seasons and localities were significantly differences (2-way ANOVA: F = 11.20, d.f. = 3, p < 0.0001 and F = 21.40, d.f. = 4 p < 0.0001).

The Mg concentration level was generally high in Worcester during summer, similar to those found at Kelderhoff. The minimum concentration level during summer was recorded at Science/UWC. The concentration level was mostly above 118.78  $\pm$  9.92 mg/kg at Grabouw during autumn. The mean concentrations of both seasons and localities were significantly differences (2-way ANOVA: *F* = 57.55, d.f. = 4, *p* < 0.0001 and *F* = 0.86, d.f. = 3, *p* < 0.0001). The high concentration of Na, 7409.84  $\pm$  399.32 mg/kg, at Kelderhoff during summer was mirrored across three of the other four sites, the exception being Grabouw, with a low concentration level of just over 3061.64  $\pm$  33.87 mg/kg ; moreover, the concentration level was mostly high, above 7409.84  $\pm$  399.32 mg/kg in Worcester during autumn. The average concentration for seasons' and localities had significant differences (2-way ANOVA: *F* = 1.72, d.f. = 3, *p* < 0.0001 and *F* = 5.66, d.f. = 4, *p* < 0.0001). Zn concentration levels were high during winter at Grabouw, but with near minimum levels were recorded at Worcester during spring, while concentration levels were above 56.11  $\pm$  5.35 at Kelderhoff during autumn. The mean concentrations of both seasons and localities were significantly differences (2-way ANOVA: *F* = 20.10, d.f. = 4, *p* < 0.0001 and *F* = 3.15, d.f. = 3, *p* < 0.0001), (Figure 3.7). Generally, the trend of mineral concentration was similar to that of *C. longus*.

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3.1.3. Correlation of soil and plant element concentration

Pearson's correlation analysis showed a positive correlation between mineral concentrations in the soils and the study species, *C. asiatica*, *C. longus* and *T. capensis*.

3.1.3.1. Correlation of soil and plant element concentration for *Centella asiatica* at different locations There was significant correlation between Zn-Fe and Zn-Mn (r = 0.873, p = 0.001, r = 0.674, p = 0.032) respectively.

3.1.3.2. Correlation of soil and plant element concentration for *Cyperus longus* at different locations The correlation of elements between the soil and *Cyperus longus*, are as follows: Grabouw during the following seasons: winter, Fe: r = -0.999; p = 0.038; autumn K r = 0.998; p = 0.036; spring Zn: r = -1, p = 0.007; Pringle Bay during autumn Zn r = 0.997, p = 0.05; Science/UWC during spring Mn: r = -1; p = 0.013, Worcester during spring Na: r = 0.999; p = 0.049; Zn: r = -0.999 p = 0.023, and summer Zn: r = 0.999, p = 0.035. Generally, all correlations were very strong as either positive or negative.

3.1.3.3. Correlation of soil and plant element concentration for *Typha capensis* at different locations The correlation of mineral elements (Ca, Fe, K, Mg, Mn Na and Zn) between the soil and *T.capensis* are as follows: Kelderhoff in spring Fe, r = 0.998, p = 0.04; Pringle Bay K and Mg were positively correlated at *r* values of 1 and 0.982 with the *p*-values of 0.014 and 0.018 for summer and winter respectively. Additionally, Science/UWC has Ca and Mg negatively correlated at r values of -1 and - 0.991 with p < 0.005 and p < 0.04 during winter and spring, while, Worcester have K and Mg positively correlated at *r* values of 0.998 and 1 with the *p* values of 0.036, 0.018 for summer and spring respectively

## 3.2. Analysis of the pH of collected soil samples

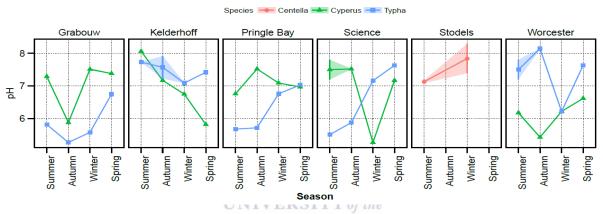


Figure 3.7: pH of the soil taken from six locations in different seasons. Each point represents mean of triplicate measurements and the bars represents plus or minus the SD.

The analysis of soil pH collected from six sites, looking at three plant species and across four seasons indicates the following:

## 3.2.1. Correlation of pH with elements in Centella asiatica soil

Large variations of the lowest pH 7.16 in winter and highest were 8.07 in summer at Stodel/Kenilworth. The Zn levels of *C.asiatica* during winter positively correlate with soil pH at Kenilworth (p < 0.01).

## 3.2.2. Correlation of pH with elements in Cyperus longus and Typha capensis soil

The lowest pH soil across the five locations and four seasons has a pH value of 5.27 in winter recorded from a soil sample collected at Science/UWC while the highest pH value of 8.07 was recorded during summer at Kelderhoff. Soil pH positively correlated with Fe levels during winter and K levels during autumn, and negatively correlated with Zn during spring at Grabouw (p < 0.05, r < -0.7). At Science/UWC, Mn positively correlated, with the soil pH during spring. A significant positive

correlation was found between soil pH and Zn levels during summer and spring at Worcester (p < p

0.05, r > 0.7).

3.3. Analysis of soil conductivity

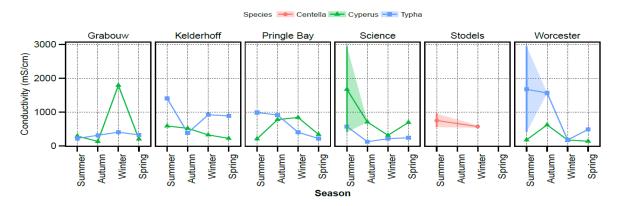


Figure 3.8: Conductivity of soil from six locations in four seasons each point represents mean and the bars represents plus or minus the SD.

The results of the soil conductivity collected from six locations, of the three plants across four seasons indicate the following:

3.3.1. Soil conductivity correlation with *Centella asiatica* 

Large variations are evident in Figure 3.8. The lowest conductivity 120.7  $\mu$ S/cm was recorded in autumn at Science / UWC and highest 1770.02  $\mu$ S/cm during winter at Grabouw (Figure 3.8).

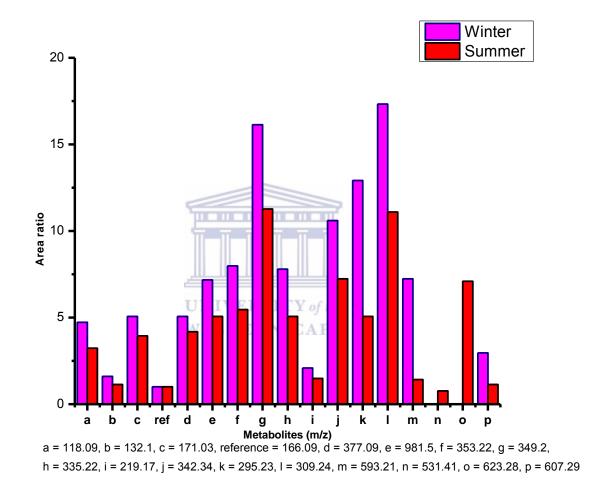
The correlation of the soil conductivity with mineral elements (Fe, Mn and Zn) in *C*.*asiatica* showed no significant difference in conductivity between seasons-518.83  $\mu$ S/cm was lowest reading during summer, while 586.61  $\mu$ S/cm was the highest reading during winter. The probability level of 0.05 conductivity for Fe has a weak positive correlate with Mn *r* - value of 0.717. Fe and Zn levels were positively correlated with soil conductivity in *Centella asiatica* (*r* < -0.7, *p* < 0.05).

## 3.3.2. Soil conductivity correlation of Cyperus longus and Typha capensis

*Cyperus longus* soil with mineral elements (Ca, Fe, K, Mg, Mn, Na and Zn) have 129.96  $\mu$ S/cm which is the lowest measurement recorded during autumn at Grabouw and the highest conductivity reading was 1770.02  $\mu$ S/cm recorded during winter also at Grabouw. There was a weak but significant correlation between K and Na concentration levels in *C. longus* and soil conductivity (*p* < 0.05). In *T. capensis*, Fe or Mg concentration levels showed significantly weak correlation with soil conductivity (*p* < 0.05).

#### 3.4. Phytochemical analysis

The phytochemical analysis of the plant material was conducted using LC-MS. The seasonal variations of the secondary metabolites earmarked as important in this study were determined using the entire plant for *C. asiatica* and *C.longus*, while only the rhizome was used for *T. capensis*. Most of the plant samples showed great variations in the concentrations of the metabolites measured across seasons as well as geographical locations (Figure 3.9 - 3.14).



#### Figure 3.9: Seasonal area ratio of Centella asiatica metabolites in Kenilworth

#### 3.4.1. Seasonal ratio of Centella asiatica metabolites

Figure 3.9 is a bar chart representing the LC-MS analysis of *C. asiatica* reflecting area variation of reference metabolites (m/z = 166.09). The other unknown metabolites are labelled as a-p. There is an indication of relatively higher quantities of metabolites during the winter season compared to summer. The most abundant metabolites of *C. asiatica* constituents are (g, j, k and l) m/z at area ratio 16.14,

10.6, 12.91 and 17.32 respectively. Furthermore, there are additional metabolites in summer (n and o with area ratio 0.76 and 7.1, respectively) which are not observed for the winter season.

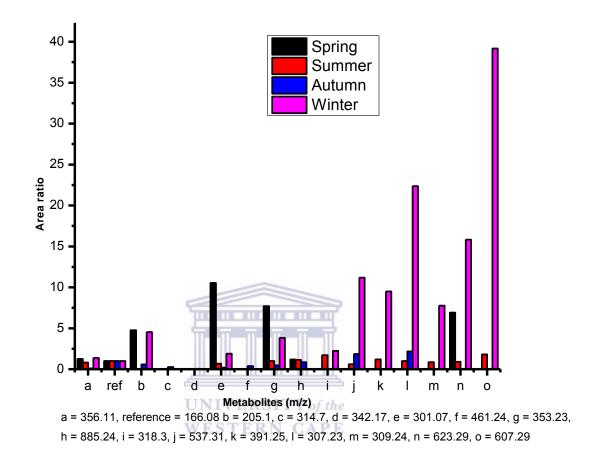


Figure 3.10: Seasonal area ratio of Cyperus longus metabolites in Grabouw.

#### 3.4.2. Seasonal ratio of Cyperus longus metabolites

Figure 3.10 is a bar chart representing the LC-MS analysis of *C. longus* using relative area ratio with reference to metabolite of m/z 166.08. The metabolites are labelled as a-o. During winter there is an indication of higher levels of metabolites [(j, k, l, m, n and o) m/z with area ratio of 11.18, 9.51, 22.35, 7.76, 15.83 and 39.18)] compared to summer, with no significant metabolite. However, in spring, unique metabolites [(b, e, g, and n) m/z with area ratio 4.7, 10.53, 7.53 and 6.9] were present that dominate the phytochemical profile of *C. longus*.

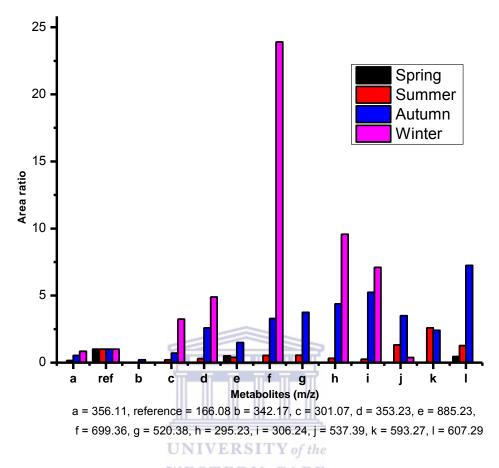


Figure 3.11: Seasonal area ratio of Cyperus longus metabolites at Pringle Bay

## 3.4.3. Seasonal ratio of Cyperus longus metabolites

The metabolite associated with m/z peak 166.08 was used as reference for comparative purposes with other metabolites by season. The results are shown in Figure 3.11, for *C. longus* from Pringle Bay. The metabolite distribution during the winter season (c, d, f, h and i) m/z [area ratios of (3.24, 4.89, 23.91, 9.57 and 7.1)] indicates a higher abundance of metabolite compared to the other seasons. The next level of abundance is during autumn with metabolites (c, d, e, f, g, h, i, j, k and I) m/z which showed the area ratios of (0.7, 2.59, 1.5, 3.28, 3.75, 4.38, 5.25, 3.5, 2.41 and 7.24) respectively. The lowest level of metabolite constituents appeared in summer (f, g, h, j, k, and l) m/z with area ratios (0.53, 0.55, 0.33, 1.33, 2.6 and 1.27). The winter season reflects higher secondary metabolites, especially when compared to the other seasons.

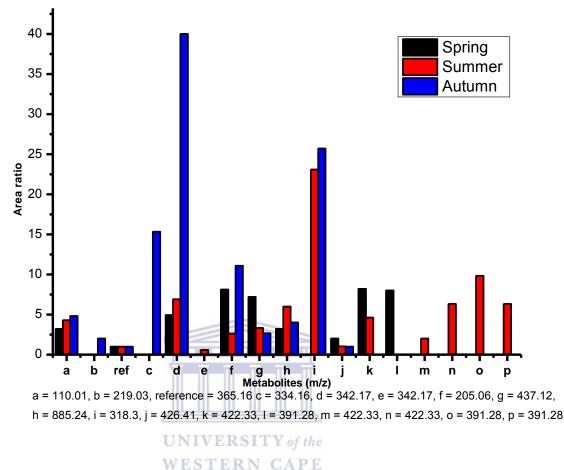


Figure 3.12: Seasonal ratio of Typha capensis metabolites from Grabouw.

#### 3.4.4. Seasonal ratio of Typha capensis metabolites

Figure 3.12 is a bar chart representing the LC-MS results of *T.capensis* using peak m/z 365.11 as reference. The highest levels of metabolites was observed during summer as expected by [(a, d, f, g, h, i, j, k, m, n, o, and p) (m/z)] with area ratio (4.29, 6.9, 2.61, 3.33, 6, 23.08, 1.03, 4.62, 2, 6.32, 9.84 and 6.32). The summer season metabolites appear to be relatively larger compared to other seasons spread throughout. The summer metabolites compared to metabolites [(a, b, c, d, f, g, h, i, and j) m/z with area ratios of 4.8, 2, 15.32, 40, 11.08, 2.67, 4, 25.71 and 1 of other seasons are significant. The metabolites which are present at low concentration are those of spring season which are (a, d, f, g, h, j, k and l) with 3.2, 4.92, 8.1, 7.19, 6, 2, 8.21 and 8.

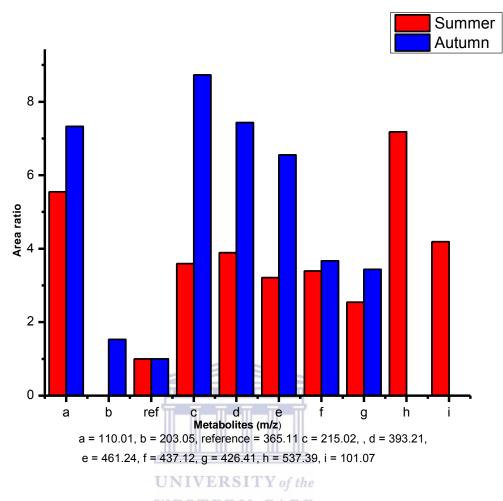


Figure 3.13: Seasonal area ratio of metabolites *Typha capensis* Pringle Bay.

3.4.5. Seasonal ratio of Typha capensis metabolites

The metabolite corresponding to m/z 365.11 was used as reference for comparison with other metabolites during season. The results for *T. capensis* are shown in Figure 3.13. The metabolites distribution for the summer season (a, c, d, e, f, g, h, and i) m/z with area ratio of (5.55, 3.59, 3.89, 3.21, 3.39, 2.54, 7.18 and 4.19) were most abundant. Autumn has the next abundant metabolites of (a, b, c, d, e, f and g) m/z with area ratio (7.33, 1.53, 8.73, 7.43, 6.55, 3.67 and 3.44) respectively.

## 3.5. Effect of geographical locations on metabolites of *Cyperus longus* and *Typha capensis*

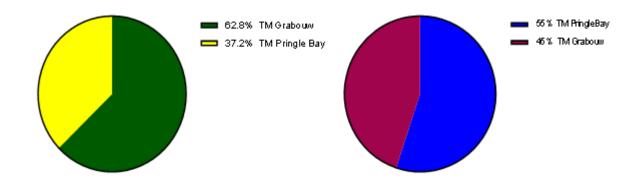


Figure 3.14: Effects of locations on quantities of metabolites.

The influence of geographical location on the relative distribution of phytochemical constituents for *C. longus* and *T. capensis* is reflected by the pie chart derived from the LC-MS results in Figure 3.14. The relative concentration of metabolites from Pringle Bay (55%) is only higher than that of Grabouw (45%) for *C. longus*. On the other hand, *T. capensis* collection shows higher levels of metabolites at Grabouw (63%) compared to Pringle Bay (37%).



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#### 4. Chapter Four Discussion

#### 4.1. Introduction

The use of medicinal plants as the first line in treatment of several ailment has become globally accepted (Pan, *et al.*, 2014). The accumulation of mineral elements in these therapeutic plants is dependent on absorption and bioavailability from soil and water. The differences in environmental factors will cause mineral elements and soil content to differ from one location to another (Chopin & Alloway, 2007; Hajar, *et al.*, 2014; Scherz & Kirchhoff, 2006). Predominantly, there are two major categories of mineral elements taken up by the xylem tissue. These mineral and their absorption is largely influenced by the conductivity and pH of the soil.

## 4.2. Soil properties

Conductivity and pH are the most common variable measured when assessing chemical properties of soil. Therefore, they were adopted as a means of investigating the absorption of mineral elements from the soil by the plants.

### 4.2.1. Soil pH

The uptake of mineral elements by plants from the soil is strongly pH dependent (Adamczyk-Szabela, *et al.*, 2015). The results show that the pH from the five locations ranged from 5.8 – 8.0 across the seasons. This depends on the data. Some sites might be acidic while other sites are basic. The fact that pH ranged from 5 to 8 across the range of sites measured does not mean that they could be both acidic and basic depending on site and season. Although this might be the case your sentence does not provide the evidence. This indicates that the soil at all sites could be both acidic and basic in character. Based on a study by Bowen & Benison (2009), pH values ranging from 5.0 to 6.9 usually indicate the absence of calcium carbonate in the soil (Hanlon, 2009). *Typha capensis* and *Cyperus longus* appear to grow better in acidic soil as the majority of the sites have a pH of below 7, with the exception of Kelderhoff and Kenilworth for *T. capensis* and *C. asiatica*, respectively. Generally, absorption increases with increasing pH and adsorption is greater at pH 6.5 than at 4.5 (Sherene, 2010). The lower the pH values the more metal can be found in the solution. As a consequence, more metal is mobilized. Mobility is therefore enhanced when there is a higher concentration of protons (pH below 5).

Brix, *et al.* (2002)'s study on root-zone acidity and how nitrogen sources affect growth and uptake kinetics of ammonium and nitrate in *Typha latifolia* L. revealed that maximum uptake rate was at pH 6.5 for NH<sup>+4</sup> and pH 5.0 for NO<sup>-3</sup>. The growth of *T. latifolia* in very acidic wetland is possible because of its ability to modify with rhizosphere conditions (Brix, *et al.*, 2002).

The season had no significant effect on soil pH, but geographic locations did and the study sites follow in order of increasing pH level: Grabouw < Pringle Bay < UWC < Kelderhoff < Worcester < Kenilworth.

#### 4.2.2. Soil conductivity

The electrical conductivity of soil is a measure of the presence of ions in the soils, the stronger the conductivity, the greater the concentration of ions in the soil available in the soil for uptake. The mean conductivity values for *T. capensis* soils were 171.57 and 2401.98  $\mu$ S/cm in winter and summer respectively at Worcester. The values for *C. longus* were 171.57 and 1770.02  $\mu$ S/cm in winter at Worcester and Grabouw respectively. The electrical conductivity of the *Centella asiatica* soil from Kenilworth was 518.83 and 586.61  $\mu$ S/cm during summer and winter. This implies that during summer more ions would be available for plant uptake at Worcester for *T. capensis*. For *C. longus* more nutrients are available in the soils at Grabouw during winter than at Worcester, while *C. asiatica* soils shows no significant difference in the amount of available nutrient between summer and winter.

## 4.3. Mineral elements in the plant

#### 4.3.1. Centella asiatica

The highest mean concentration of the Fe was found in *C. asiatica*  $(231.08 \pm 28.90 \text{ mg/kg})$  and was higher in concentration than any other trace element recorded. Fe is an element essential in the human body for the production of haemoglobin and in the oxygenation of red blood cells. It is necessary for a healthy immune system and energy production (Singh, *et al.*, 2015). Several studies have shown how Fe availability correlates with the bactericidal action of lactoferrin and lysozyme, killing Gramnegative bacteria (Kanwar, *et al.*, 2015). The value of Fe to the immune system has been recorded in medical literature; the use of *C. asiatica* in the treatment of skin diseases in the traditional practices might then be attributed to the considerable amounts of Fe present. Moreover, Fe and Mn are important for the activation of several essential enzymes that are key to the full utilization and digestion of foods, regulation of some vitamins during energy production, and essential for the control of body reactions to amino acids breakdown (Renthlei, *et al.*, 2016).

In mitochondria, Mn is an essential part of the antioxidant resistance structure used to defend against harmful free radicals produced by injured skin cells (Renthlei *et al.* 2016). It is also a constituent of manganese superoxide dismutase (Renthlei, *et al.*, 2016). The manganese concentration recorded in the present study ranges from  $(45.43 \pm 22.51)$  to  $(220.13 \pm 4.42)$  mg/kg, with *C. asiatica* having the highest concentration. In light of the role Mn has in the antioxidant resistance system and immune

responses, the traditional use of *C. asiatica* for treatment of skin diseases may be ascribed to the high concentrations of Mn these plants store.

The various biochemical reactions involved in metabolism are Zn dependent, and Zn is mostly available in all animal and plant tissues. Zinc has an important role in the maintenance of healthy skin by activating and replenishing the body's cells (Rahman, 2007). The presence of Zn metalloenzymes enhance the stability of cell membranes, T- cell mediated response, immune response improvement, is involved in cell and tissue growth, metabolism and tissue restoration (King, *et al.*, 2016).

The appreciable presence of Zn concentration  $(7.930 \pm 1.238)$  to  $(18.209 \pm 5.493)$  mg/kg in *C. asiatica* is the highest concentration of Zn recorded in this study. The use of *C. asiatica* in appropriate quantities for treating skin diseases in the traditional practices can be understood when taking into account the comparatively high levels of Zn in this wetland species. Preliminary assessment of nutritional value of traditional leafy vegetables by Odhav, et al., (2007) in KwaZulu-Natal University also identified Zn, Fe and Mn in a significant quantity. However, Chizzola, (2012) in a review paper detected only Zn and Fe in the leaves and roots of *C. asiatica*.

## 4.3.2. Cyperus longus

*Cyperus longus* contains four macro elements (Ca, K, Mg and Na) and three microelements (Fe, Mn and Zn) that are most essential for the cure of stomach cramp, colds and diaphoresis. The results of the present study revealed that across the five locations and throughout four seasons *C. longus* had the highest concentration of the micro- and macro-elements found in the soil incorporated into their tissues, with the exception of K Figure. 3.5.

The results obtained show that the highest concentration of Calcium in *C. longus* was recorded at Science/UWC during autumn and compared with soil concentrations of calcium from Grabouw during winter; Calcium plays a vital role in maintenance of strong bones, teeth and human blood Figure 3.5. In addition, Ca is important for cardiac muscle performance, coagulation of milk, permeability of cell regulation and clotting of blood (Nordin, 2013; Shanahan, *et al.*, 2011). The deficiency of calcium leads to back pain, indigestion, osteoporosis, rickets and indigestion (Shanahan, *et al.*, 2011).

Mean Calcium concentration in the soil was highest at Grabouw (10415 mg/kg) during spring, while the lowest mean concentration was recorded at Pringle Bay (1570.65 mg/kg), also during spring. However, the highest plant concentration, 7069.38 mg/kg, was found at Kelderhoff during spring and

the lowest, 36.62 mg/kg, was also recorded at Kelderhoff, this time during winter. This implies that Calcium concentration in the soil did not correlate with the concentration in the plants. Calcium is important for protection and improvement of the skeleton and also has vital roles in cardiac and neuromuscular function as well as the development of bones and teeth (Perveen, et al., 2012). The presence of Ca may also affect Na requirement (Ahmad, et al., 2016). Iron is the main component of haemoglobin, myoglobin and other haemo-proteins, many of which are enzymes of the Krebs cycle (Fraga & Oteiza, 2002). Iron deficiency also causes neurodegenerative conditions and anaemia (Shirin, et al., 2010). Iron is the most available, and most important, component of all animals and plants (Lukiw, 2015). The results of this study indicate that the lowest soil concentration of Fe (36.57  $\pm$  33.94 mg/kg) was recorded during spring at Pringle Bay while the highest soil concentration of Fe  $(4220.62 \pm 713.24 \text{ mg/kg})$  was recorded at Worcester, also during spring. In the plant, the concentration of Fe is in the range of  $40.22 \pm 20.21$  mg/kg in autumn at Worcester and  $235.62 \pm 40.69$ mg/kg during summer at Grabouw. The recommended level of Fe in medicinal plant is 20 mg/kg, while its dietary intake is 10 - 28 mg/day (WHO, 1998). The high concentrations of Fe in the plant may be due to high amount of Fe in the soil, as well as be due to high root absorption capacity in seasons when Fe is readily available for absorption from the soil.

#### 4.3.3. Typha capensis

*Typha capensis* is an indigenous medicinal plant found in wetlands of several provinces throughout South Africa, especially in Western Cape. Although numerous studies have been conducted in South Africa, with inconsistent reports on the evaluation of different elemental composition of *T. capensis*, there is no scientific evidence to support its traditional use as an agent of female conception, easy parturition and a libido booster (Abdillahi & Van Staden, 2012; Henkel, *et al.*, 2012; Hutchings, *et al.*, 1996; Watt & Breyer-Brandwijk, 1962).

El-Ameir (2013) and Collins, *et al.* (2005) have reported that *T. capensis* contains four macro elements (Ca, K, Mg and Na) and two micro elements (Fe and Zn) that are considered essential nutrients and have reportedly been used for the possible treatment of sexual dysfunctions, such as infertility and sexual performance enhancement. The results of the analysis revealed that across the five locations and throughout the four seasons, *T. capensis* generally absorbed almost all of the macro- and micro-elements available in the soil into its tissues. The only exceptions to this soil-tissue correlation were high concentrations of Ca in the soil in Grabouw during spring, Kelderhoff in winter, Pringle Bay during winter and summer, and Worcester throughout the seasons which did not show up in the tissues of *T capensis* as shown in (Appendix 2). The presence of high concentrations of Ca in the soil at Grabouw may be due to accumulation from the surrounds and the nature of the parent rock

making up the locations (Marschner, 2011; White & Brown, 2010). There is a positive correlation in Ca concentrations between the soil and plant at UWC during spring. The high concentrations of Ca in *T. capensis*, which ranged between 509.49 mg/kg - 6952.93 mg/kg, may be responsible for its penile erectile functions; the Ca<sup>2+</sup> calmodulin pathway has a vital function in triggering the sexual urge in males (Samuel, et al., 2016). In addition, the dietary recommended intake (DRI) of Ca is an average daily allowance in the range of 1300 mg – 2500 mg/kg (Food & Board, 2004; Means, 2013; Samuel, *et al.*, 2016).

*Typha capensis* has a good ability to accumulate Ca in its tissues as demonstrated in Appendix 4. This is important because of the role it plays in penile erection, sustenance or triggering sexual desire in males, heart and muscles functioning, bones and teeth formation (Johnston, 2005; Samuel, *et al.*, 2016; Soetan, *et al.*, 2010; Underwood, 2012; Vaskonen, 2003). Several biochemical and physiological processes such as muscle coupling, contraction and excitation, spermatozoa motility, nerve excitable regulation, egg fertilization and cell reproduction require calcium (Nordin, 2013).

The highest annual mean soil and plant concentrations of Mg were detected at Kelderhoff while the lowest annual mean soil and plant concentrations were detected at UWC (value). (Appendix 2). The implication is that the concentration of Mg in the tissue of *T. capensis* is directly proportional to the concentration of Mg in the soil. Moreover, there is a significant correlation between Mg in the soil and plant tissue of *T. capensis* at all locations. The differences in the elemental concentration in plant can be attributed to the differences in the locations where the plants were collected (Ajasa, *et al.*, 2004). The recommended daily intake (DRI) of Magnesium is between 65 mg/day – 350 mg/day. *Typha capensis*, however, has a relatively low Mg concentration (79.19 mg/kg) at UWC during summer, compared to Worcester (324.44 mg/kg) in the same time period as shown in (Appendix 4). The high Mg concentration levels in *T. capensis*, specifically in Worcester, could enhance the production of androgen, estrogens, hormones and neurotransmitters through numerous mechanisms to trigger sexual desire in males. Magnesium is an active component of numerous enzymes systems in which thymine pyrophosphate is a co-factor (Aremu & Ibrahim, 2014; Samuel, *et al.*, 2016; Soetan, *et al.*, 2010).

The concentration of Na in *T. capensis* for all five locations and all seasons was higher compared to the soil Na concentrations. This may be due to the accumulation of Na by the plant rhizome from the soil, even when Na is present at low concentrations. In addition, the bioavailable elements in the soil are readily mobile (Olowoyo, *et al.*, 2012; Sherene, 2010; Tangahu Bieby Voijant, *et al.*, 2011). Potassium is another important element for which concentration level (10654.73  $\pm$  89.81 - 25045.03  $\pm$ 

3963.27, Appendix 4) was generally high in plants, through all the seasons and locations when compared to the soil concentration. Potassium and sodium are involved in maintenance of human body tissues excitability, ionic balance, gastric juice formation in the stomach and contraction of muscles. The erectile function in penile tissue is enhanced with K induction on vascular smooth muscle (Samuel, et al., 2016; Soetan, *et al.*, 2010; Vimala & Shoba, 2015). The K recommended daily intake is 2300 mg/day.

Iron concentrations in the soil differ at Grabouw, Pringle Bay, and Worcester during autumn, spring and winter Figure 3.6. The formation of haemoglobin requires Fe; haemoglobin has an important role in transportation of oxygen in normal functioning of human central nervous system, and electron movement around the body through oxidation of carbohydrates, fats and proteins. Deficiency of iron in human blood causes anaemia (Bhowmik & Badal, 2012). The Zn concentration level in the rhizome was higher than soil concentrations at all locations and seasons, especially at Grabouw during winter. Zinc is an essential plant micronutrient that is involved in several physiological roles; for example, when plants are Zn-limited it leads to reduced crop production (Sian, et al., 2002). The most widespread micronutrient deficiency is Zn shortage. Beside its functions in crop yield, Zn also has a role in the primary functioning of cellular processes in all living organisms and is concerned with restoring the human immune system after illness; insufficient intake of Zn causes the human body to experience hair and memory loss, skin problems and muscles weakness (Brown, et al., 1993; Hafeez, et al., 2013). Zn availability is mostly seasonal from findings Zinc is abundant during summer. Assessing the effect of seasonal changes on the quantity of plant metabolites is an important step to establish the most suitable harvesting season to ensure plant tissue is concentrated with high amounts of metabolites.

#### 4.4. Effect of season and location on secondary metabolite levels

In this section, the influence of season and locality on metabolite concentration in *C. asiatica*, *C. longus* and *T. capensis* is discussed. The seasonal variation of phytochemical constituents in the study species were investigated by comparing their LC-MS profiles across different seasons -[*C. asiatica* (summer and winter), *C. longus* (autumn, spring, summer and winter) and *T capensis*- (autumn, spring and summer)]. Additionally, using the LC-MS profiles for each location, geographic variation of phytochemical constituents across the locations can be assessed. Secondary metabolites have important medicinal applications (Akula & Ravishankar, 2011) and the accumulation of these secondary metabolites in medicinal plants generally takes place due to stress from environmental conditions.

The resolution bar chart of LC-MS results of secondary metabolites in C. asiatica and C. longus (Figures 3.9, 3.10 and 3.11) shows the highest quantity in the winter season. Meanwhile, highest secondary metabolites were present during summer in this investigation of the secondary metabolites in T. capensis across the summer, autumn and spring seasons (Figures 3.12 and 3.13). The implication of this is that the secondary metabolites are more abundant during the winter for C. asiatica and C. longus and during summer for T. capensis. Consequently, the best harvesting period for C. asiatica and C. longus is in winter while T. capensis should be harvested in the summer. There is high possibility of low nutritional/ medicinal values in the autumn and spring harvests as the least number of secondary metabolites was measured during these seasons. This harvesting approach is supported by Brasileiro, et al. (2015) in their investigation of the influence of planting and harvesting times on the total phenolic content and antioxidant activity of *Talinum triangulare* (Jacq.) Willd. They reported that the winter could favour more production of secondary metabolites and consequently made this a suitable harvesting time for medicinal application. de Lazzari Almeida, et al. (2016), also suggested that winter is the best season for collecting leaves of Mikania laevigata (Schultz) in order to increases concentration of coumarins and/or caffeoylquinic acid. The high quantity of metabolite yield in the autumn, as reported by these studies, is justified by Afonso, et al. (2015) in their investigation of the significant effect of harvesting season on the relative abundance of the metabolites. They conclude that autumn is the best for the achievement of high quantity metabolites. However, as stated above, winter and summer are significantly favourable to production of high quantities of metabolites in the study species selected for this investigation, and therefore, harvesting should occur in those seasons.

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4.5. The effect of seasonal changes on the quantity of plant metabolite and its significance for establishing the suitable harvesting season

According to Figure: 3. 14. the results of the current study, there is variation in the seasonal quantities of metabolites in the three wetland medicinal plants. Additionally, results showed that the *C. asiatica*, *C. longus* and *T. capensis* each possess several varieties of secondary metabolites as reported by (Gololo, *et al.*, 2016).

Environmental/abiotic factors, whether directly or indirectly, influence the elemental accumulation in medicinal plants and consequently the quality and quantity metabolites (Liu, *et al.*, 2015). The examples of these environmental factors are altitude, precipitation, temperature, salinity and sunshine. The roles of these physical factors on the formation of metabolites in medicinal plant, as documented in the literatures, are highly significant (Akula & Ravishankar, 2011). Therefore, the current study is a justification of the previously published articles on the influence of the environment on the quantity of metabolites in the medicinal plants. Dong, et al. (2011) reported the influence of altitudes and

temperature on the accumulation of flavonoids and chlorogenic acid. They also state that sunshine and temperature promoted the production of geniposidic acid. Likewise, Figueiredo, et al. (2008) and Ganzera, et al. (2008) reported the environmental influences on the secondary metabolites in *Matricaria chamomilla* cv. specific plant species harvested from different locations showed environmental effects as reported.

The influence of environmental stress on the quantity of metabolites produced by medicinal plants cannot be overlooked. Plants appear to responds to stress by altering the secretion of certain chemical compounds which may, in the long run, affect the quantity of its metabolites. This may lead to either an increase or a decrease in the metabolite yield of medicinal plants (Gorelick & Bernstein, 2014). *Cyperus longus* is evidently less sensitive to stress, as metabolite production in Grabouw (45%) is only slightly lower than that of Pringle Bay (55%).

# The influence of geographic location on metabolites quantities shows the variation of secondary metabolites under different climatic conditions.

*Cyperus longus* from Pringle Bay, grew in low altitude conditions (18m above sea level) which likely favoured the production of secondary metabolites (55%), while a 45m increase in altitude for *C. longus* growing in Grabouw resulted in a 10% lower metabolite production yield., Conversely, for *T. capensis* growing at high altitude (63m above sea level) in Grabouw (as found in Table 2.1), strongly favoured the production of secondary metabolites 63%, while the Pringle Bay *T. capensis* specimens produced only 37%. Ganzera, *et al.* (2008) reported that plants, such as *Matricaria chamomilla*, that grow at higher altitudes exhibit an increased production of phenolics compared to their lower altitude counterparts. It has been reported that the highest percentage of essential oils constituents was found in *Stachys lavandulifolia*, growing at the highest altitude of 3200m above sea level compared to 2400 and 1600m above sea level (Mahzooni- Kachapi, *et al.*, 2014). The influence of altitude on metabolite production in plants is multi-faceted as changes in altitude can change the availability of water to plants' root, temperature changes, relative humidity, rates of sunlight and speed of wind.

## 5. Chapter Five Conclusion

This study examined the elemental and metabolite composition of three medicinal, wetland plant species, *Centalla asiatica*, *Cyprus longus* and *Typha capensis*, to determine the influence of geographic location and seasonal change on elemental and phytochemical composition. Significant

concentrations of elements including Na, Ca, K, Mg, Mn, Fe and Zn, were detected in the soil at all five study locations, with Fe having the highest concentration overall. The plants in these locations contained significant concentrations of Ca, Fe and Na. which suggests that there is a direct relationship between the elemental composition in the soil and that of the medicinal plants in the studied locations.

The concentrations of Ca, K and Na in the plants were significantly higher than the concentration of these elements in the soil. This may be due to the fact that these elements are retained in the tissues of these plants after being taken up from the soil. However, Fe level is higher in the soil compared to the plants.

Seasonal change significantly influences the concentration of elements in both the soil and the plants. The soil showed high concentrations of Ca and Fe in spring, while Na was high during autumn. Similarly, the plants showed high concentrations of Ca and Na in spring and autumn, respectively. Interestingly, the high plant concentrations of K in summer suggest that uptake and storage of K exceed that of Fe in this season. In general, the study species directly absorbed and retained more of Ca, K and Na during these periods of the year when these elements were in good supply in the soil.

In general, soil from urban locations (Kelderhoff and UWC-science) had higher elemental concentrations compared to those of rural (Pringle Bay and Grabouw) and natural (Worcester) areas. This could be attributed to a number of factors, including f anthropogenic activity and differences in the geology of these different locations.

The study species each produced different types of secondary metabolites, which would have unique biological functions. The use of LC-MS analytical techniques showed that during the different seasons there were significant differences in the relative concentrations of the metabolites in the wetland medicinal plants investigated. The relative abundance of metabolites peaked at different seasons for each of the analysed plants. *Centella asiatica* and *Cyperus longus* peaked during winter while *C. longus* also produced an abundance of metabolites at Pringle Bay in autumn. Finally, for *Typha capensis*, the accumulation of metabolites was highest during summer at Grabouw and Pringle Bay.

The extracts obtained from *Centella asiatica* in winter contain significant constituents of particular metabolites making this the most favourable harvesting season for the plant. Harvesting *Cyperus* 

*longus* between autumn and winter would be the ideal as metabolite concentrations peaked during those seasons. Meanwhile, *Typha capensis* metabolites concentrations were highest in summer at Grabouw and Pringle Bay and consequently this would be the ideal season for harvesting. The concentrations of metabolites varied from one location to another for each species. A slight increase in metabolites in *C. longus* at Pringle Bay compared to Grabouw could be a means to cope with stress at the location. , *T. capensis*, on the other hand, had a stronger response to the increased altitude, producing significantly more metabolites at Grabouw compared to Pringle Bay. The above results show that specimen collection period (i.e. which season collections are made) is an important factor to be considered when conducting phytochemical studies on the biological activity of the plants.

In Africa, the trade of medicinal plant is of vast economic importance. Ghana sold over 900,000 kg valued at US\$7.8 - \$15 million from the domestic major city markets in Takoradi (Leonti, 2013). In comparison, the reported profit from the sale of medicinal plants in South Africa is within the range of US\$0.16 - 0.34 million. In this study, it was found that both summer and winter are appropriate seasons for abundant metabolites production from *Centella asiatica* and *Cyperus longus*, moreover, summer is the appropriate season for abundant metabolites production in *T. capensis*.

#### 5.1. Recommendation

The advantages of traditional medicine, among which are accessibility, affordability, diversity and flexibility are incomparable with other, orthodox medicines (Ncube, 2010). In South Africa, the government has adopted traditional health care into its health care system. Although it has some challenges to overcome, such as lack of scientific evidence pertaining to the efficacy of several of traditional medicinal therapies, traditional medicinal can be standardized through national policy and the regulatory frameworks that define access, safety, efficacy and quality.

The analytical studies conducted on the three medicinal, wetland plants revealed that they are good sources of important consumable elements and may contribute significantly to the recommended dietary intake for most individuals. These elements include the following in order of decreasing importance: K, Ca, Na, Mn, Mg, Fe and Zn. This study confirms that consumption of these important wetland plants could be useful to the rural dwellers and, possibly, the entire nation. For centuries, indigenous communities have been using these plants as herbs without any scientific verification of the medicinal or elemental constituents. These benefits could be enhanced if the government provides additional resources to research medicinal plants identify their medicinal constituents and then safely extract the active ingredients for industrial production.

The South African government should consider a comprehensive ethnobotanical mapping exercise to assess the abundance and diversity of medicinal plants in all habitats within its borders. At present this valuable resource is vulnerable to chaotic, unscientific, unsystematic exploitation and an up-to-date record of indigenous herbs/plants and their medicinal uses should be produced.

The government of South Africa should invest in educating wild harvesters about sustainable harvesting techniques and the best season to harvest without causing adverse effects on the diversity. Harvesting sustainably in the appropriate season will yield better quality plant products. Both rural and urban communities in South Africa should be enlightened on the health benefits of medicinal plants and incorporate the plants for pharmaceutical adoption. Ideally, the development and implementation of conservation legislation to prevent over-harvest of the plants should take place before harvesting begins. The Department of Health should work in synergy with traditional herbal practitioners in accordance with the country and global health policies. Laboratory scientists should use accurate modern equipment for authentication of plant species, chemical analysis, metal contaminant tests and microbial laboratory test. Eradication of the adulteration of South Africa (Junaid

& Nasreen, 2012).



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#### APPENDIX

			Parameters for (	Cyperus longus	soil				
	Ele								
Season	Ca	Fe	K	Mg	Mn	Na	Zn	Location	
Winter	6967.13 ± 343.48	$132.66 \pm 131.02$	$11.19 \pm 6.75$	$19.28\pm4.61$	$1.41 \pm 0.72$	$0.00 \pm 0.00$	$1.44 \pm 1.29$		
Spring	$10415.24 \pm 437.00$	3989.58 ± 1281.36	$137.37 \pm 42.13$	$61.64 \pm 5.10$	$29.87 \pm 3.20$	$164.37 \pm 81.83$	$38.25 \pm 17.07$	8	
Summer	7863.96 ± 840.05	1990.79 ± 588.03	$50.06 \pm 18.86$	$51.69 \pm 6.23$	$26.37 \pm 1.79$	$3498.53 \pm 713.17$	$17.06 \pm 1.79$	Grabouw	
Autumn	8541.11 ± 595.13	$453.25 \pm 281.10$	$53.12 \pm 15.41$	$42.80 \pm 6.45$	$7.08 \pm 3.19$	$1891.14 \pm 110.22$	$12.21 \pm 4.15$	hrab	
f	6.25	5.97	4.68	10.27		20.56	3.05	0	
р	0.02	0.02	0.04	0		0	0.09		
Winter	7226.18 ± 700.57	2430.52 ± 1118.90	$135.36 \pm 34.62$	$62.88 \pm 5.10$	$30.19 \pm 3.43$	$0.00 \pm 0.00$	$24.58 \pm 3.25$		
Spring	$3274.10 \pm 332.80$	$3100.36 \pm 1448.94$	$253.09 \pm 65.68$	$50.70 \pm 7.37$	$28.24 \pm 4.12$	$164.37 \pm 81.83$	$22.26 \pm 7.62$	벌	
Summer	3054.45 ± 718.58	$262.51 \pm 86.39$	$171.00 \pm 17.41$	$46.75 \pm 3.63$	$20.04 \pm 0.87$	3498.53 ± 713.17	$19.42 \pm 3.54$	Kelderhoff	
Autumn	7535.88 ± 86.58	54.57 ± 29.79	$325.64 \pm 2.99$	$221.46 \pm 6.52$	$2.61 \pm 0.47$	$1891.14 \pm 110.22$	$7.04 \pm 0.85$	eld	
f	21.15	2.8	5		14.14	4.62	2.98	Ř	
р	0	0.11	0.03		0	0.04	0.10		
Winter	2834.27 ± 322.77	$0.00 \pm 0.00$	84.14 ± 12.61	$15.36 \pm 1.43$	$0.00 \pm 0.00$	$72.00 \pm 48.43$	$3.93 \pm 0.81$		
Spring	$1570.65 \pm 827.58$	36.57 ± 33.94	$12.28 \pm 7.30$	$3.87 \pm 3.87$	$0.00 \pm 0.00$	$48.00 \pm 48.0$	$30.42 \pm 7.19$	ay	
Summer	3953.74 1290.51	564.11 ± 489.23	$62.44 \pm 17.00$	$15.25 \pm 4.84$	$2.84 \pm 1.54$	$2165.77 \pm 360.20$	$1.27 \pm 0.87$	Pringle Bay	
Autumn	6334.87 ± 329.30	804.20 ± 475.05	$145.15 \pm 26.99$	$46.03 \pm 2.33$	$1.10 \pm 0.66$	$1061.12 \pm 532.81$	8.53 ± 1.13	ing	
f	6.39	1.35	9.85	28.52		9.64	12.93	Pr	
p	0.02	0.33	0.01	0.00		0.01	0.00		
Winter	3646.70 ± 1236.40	$242.78 \pm 242.78$	$239.74 \pm 6.31$	$61.27 \pm 50.87$	$0.00 \pm 0.00$	594.49 ± 594.49	$24.58 \pm 19.54$	D D	
Spring	$2871.56 \pm 1126.99$	$1167.50 \pm 775.83$	$323.32 \pm 282.39$	$63.97 \pm 50.39$	$5.29 \pm 1.94$	$1557.15 \pm 252.07$	$21.31 \pm 17.82$	8	
Summer	$2537.72 \pm 179.13$	724.64 ± 179.12	374.97 ± 34.20	$131.84 \pm 8.58$	$5.96 \pm 2.19$	$201.36 \pm 104.56$	$51.38 \pm 2.31$	Science or UWC	
Autumn	5568.76 ± 190.37	$0.00 \pm 0.00$	398.79 ± 32.81	$239.74 \pm 6.31$	$3.26 \pm 1.74$	$4402.03 \pm 570.01$	$2.16 \pm 1.18$	106	
f	2.57	0.63	0.05	5.34	0.51	19.10	2.33	ciei	
р	0.13	0.62	0.98	0.03	0.69	0.00	0.15	x	
Winter	975.93 ± 518.21	2731.43 ± 602.36	$131.54 \pm 53.28$	$32.96 \pm 4.02$	$27.97 \pm 1.16$	1832.65 ± 961.46	$18.93 \pm 2.10$		
Spring	2455.03 ± 817.05	4220.62 ± 713.24	$219.20 \pm 129.64$	$68.19 \pm 38.24$	$42.51 \pm 13.11$	$1303.12 \pm 761.24$	33.99 ± 16.19	L.	
Summer	$1975.16 \pm 480.85$	4150.07 ± 770.34	$315.92 \pm 116.79$	$85.50 \pm 33.20$	$42.71 \pm 11.37$	$0.00 \pm 0.00$	$49.83 \pm 15.62$	Worcester	
Autumn	3284.15 ± 523.71	$2434.68 \pm 986.89$	$955.58 \pm 145.08$	$171.85 \pm 9.32$	$13.29 \pm 5.28$	253.88 ± 99.46	$56.57 \pm 2.88$	lord	
f	2.58	10.63	10.32	5.22	5.09	1.98	2.19	M	
p	0.13	0.00	0.00	0.03	0.03	0.20	0.17		

# Appendix 1: Elemental concentrations for *Cyperus longus* soil (mean $\pm$ S.D) (mg/kg)

			Parameters for Typ	ha capensis soi	1				
	Elemental concentration for <i>Typha capensis</i> soil (mean ± S.D) (mg/kg)								
Season	Ca	Fe	Mg	Na	Zn	Location			
Winter	$0.15 \pm 0.15$	8.97 ± 8.97	$214.46 \pm 18.53$	$38.91 \pm 4.62$	63.98 ± 20.37	$73.91 \pm 72.29$			
Spring	$3574.17 \pm 324.16$	$0.00 \pm 0.00$	$101.84 \pm 22.54$	$19.67 \pm 0.98$	55.68 ± 17.99	$4.48 \pm 2.58$	*		
Summer	$1150.27 \pm 515.04$	$5197.91 \pm 2469.42$	$251.31 \pm 15.19$	66.75 ±1.46	1627.57±1338.37	$34.37 \pm 2.10$	100		
Autumn	$1435.04 \pm 595.85$	$799.62 \pm 416.05$	$574.58 \pm 77.12$	$82.15 \pm 8.34$	101.38 ± 12.53	$29.06 \pm 14.82$	Grabouw		
f	12.26	3.96	23.43		1.35	0.61	5		
р	0.00	0.05	0.00		0.33	0.63			
Winter	5422.52 ± 3036.59	$236.07 \pm 236.07$	$5237.14 \pm 154.81$	$417.10 \pm 17.02$	548.00 ± 118.07	$30.70 \pm 14.64$			
Spring	$0.00 \pm 0.00$	760.93 ± 678.25	$4029.04 \pm 279.71$	$345.80 \pm 25.72$	499.04 ± 95.52	19.66 ± 10.09	Ħ		
	$5652.16 \pm 1406.97$	983.98 ± 971.36	2648.67 ± 1281.88	$280.71 \pm 79.00$	689.65 ± 34.25	$12.65 \pm 7.81$	Kelderhoff		
Autumn	3166.68 ± 162.24	$1447.78 \pm 727.80$	$1655.90 \pm 65.23$	$249.85 \pm 13.58$	$12.16 \pm 6.36$	$12.16 \pm 6.36$	alde		
f	2.46	0.51	5.62	2.99	3.67	0.72	Ň		
р	0.14	0.69	0.02	0.10	0.06	0.57			
Winter	7649.52 ± 591.25	4115.86 ± 648.14	$113.42 \pm 5.89$	$62.00 \pm 3.88$	2896.21 ± 227.98	$9.90 \pm 0.45$			
Spring	$3274.72 \pm 1644.84$	$18.90 \pm 18.90$	62.87 ± 33.76	$21.35 \pm 2.21$	1507.53 ± 332.58	$1.97 \pm 1.04$	ay		
Summer	$4980.02 \pm 675.21$	$21.79.58 \pm 1228.57$	$206.30 \pm 28.65$	$16.71 \pm 4.11$	734.67 ± 632.27	$1.77 \pm 1.08$	Pringle Bay		
Autumn	$70.69.26 \pm 429.36$	$147.78 \pm 727.80$	$448.97 \pm 9.76$	$34.86\pm2.18$	$113.01 \pm 11.98$	$6.65 \pm 1.86$	1		
f	4.35	4.97			10.24	10.46	Pri		
р	0.04	0.03			0.00	0.00			
Winter	$10591.76 \pm 599.24$	$157.80 \pm 80.53$	$394.37 \pm 26.83$	$0.97 \pm 0.89$	$0.00 \pm 0.00$	$8.99 \pm 8.71$	ce		
Spring	$3437.05 \pm 1155.89$	$123.87 \pm 64.46$	$297.54 \pm 130.63$	$4.05 \pm 4.05$	$1442.57 \pm 724.55$	$1.28\pm0.64$	ien		
Summer	$4813.20 \pm 410.21$	$194.08 \pm 171.74$	$297.54 \pm 130.63$	$53.07 \pm 23.62$	1725.48 ± 108.07	$19.62\pm18.24$	UWC or Science		
Autumn	$6562.69 \pm 1340.43$	$159.96 \pm 159.96$	$617.04 \pm 66.05$	$62.85 \pm 12.51$	$1600.85 \pm 74.17$	$6.31 \pm 6.31$	5		
f	10.52	0.05	3.42	5.70	4.76	0.53	M		
p	0.00	0.98	0.07	0.02	0.03	0.67	þ		
Winter	$7050.98 \pm 371.63$	$514.28 \pm 255.97$	$97.47 \pm 6.35$	$24.17\pm3.40$	$1615.60 \pm 9.94$	$44.32 \pm 16.30$			
Spring	$2615.95 \pm 1343.50$	$15.05 \pm 9.45$	$43.29 \pm 21.54$	$28.99 \pm 4.97$	$1021.03 \pm 308.65$	$0.00\pm0.00$	5		
Summer	$7543.13 \pm 3522.99$	$1256.62 \pm 625.67$	$1560.92 \pm 1332.54$	$210.53\pm75.32$	$813.90 \pm 100.97$	$10.72\pm5.22$	Worcester		
Autumn	$10319.85 \pm 1855.78$	$36.65 \pm 34.24$	$397.14 \pm 149.84$	$78.02\pm30.48$	$297.28 \pm 148.99$	$2.84\pm2.55$	or		
f	2.28	2.95	1.12	0.04	9.31	5.57	ă _		
p	0.16	0.10	0.40	4.55	0.01	0.02			

**Appendix 2:** Elemental concentrations for *Typha capensis* soil (mean ± S.D) (mg/kg)

			Parameters for Cyp	erus longus pla	nt			
	Ele							
Season	Ca	Fe	K	Mg	Mn	Na	Zn	Location
Winter	$704.66 \pm 385.43$	$80.62 \pm 6.95$	$7396.46 \pm 72.74$	$118.38\pm4.37$	$237.85 \pm 1.08$	$1965.16 \pm 72.23$	$179.54\pm1.27$	
Spring	$1074.02 \pm 991.68$	$233.77\pm6.53$	$11503.94 \pm 138.16$	$136.79\pm3.91$	$224.71\pm2.25$	$2598.32 \pm 70.73$	$133.65\pm1.54$	₿
Summer	$3475.15 \pm 1116.49$	$235.62\pm40.69$	$11324.02\pm 339.32$	$140.20\pm6.15$	$220.33\pm5.00$	$2428.19 \pm 65.98$	$131.18\pm3.42$	noc
Autumn	$2848.73 \pm 1095.55$	$130.51\pm10.07$	$10548.26 \pm 36.17$	$180.81\pm3.28$	$124.13\pm2.56$	$3143.20 \pm 45.75$	$39.33 \pm 1.83$	Grabouw
f	2.02	12.93						0
р	0.19	0.00						
Winter	$36.62 \pm 36.62$	$174.97\pm5.87$	$13829.86 \pm 228.05$	$236.58\pm4.37$	$237.85\pm1.08$	3611.70 ± 82.49	$115.31 \pm 37.12$	
Spring	$7069.38 \pm 1375.94$	$140.78\pm6.97$	$10587.38 \pm 174.42$	$289.42\pm2.73$	$62.64 \pm 1.04$	$4447.48 \pm 73.13$	$63.96 \pm 1.32$	벌
Summer	$1354.13 \pm 826.80$	$108.81{\pm}\ 14.10$	$11811.31 \pm 98.30$	$286.22\pm5.43$	$66.91\pm0.81$	$4064.48 \pm 24.29$	$75.18 \pm 1.04$	srhe
Autumn	$5016.18 \pm 1431.64$	$70.15 \pm 5.79$	$8078.68 \pm 41.01$	$144.82\pm2.29$	$146.02\pm0.70$	$2134.64 \pm 41.27$	$104.02\pm1.19$	Kelderhoff
f	9.10	25.41					1.67	м
p	0.01	0.00					0.25	
Winter	$1034.82 \pm 21.28$	$168.43 \pm 20.63$	8632.62 ± 2723.68	$188.70 \pm 25.87$	$112.63 \pm 38.13$	3596.43 ± 418.69	$110.13 \pm 21.28$	
Spring	$6408.01 \pm 1359.77$	$168.21\pm4.06$	$10982 \pm 1202.40$	$234.31\pm37.92$	$152.03 \pm 6.38$	$3655.67 \pm 1112.20$	$123.57 \pm 21.20$	ay
Summer	$115.06 \pm 813.44$	$130.03 \pm 12.03$	$10329.26 \pm 186.86$	$243.43\pm 6.12$	$14.74 \pm 2.09$	$4713.43 \pm 100.88$	$71.09 \pm 1.92$	Pringle Bay
Autumn	$2006.90 \pm 1015.59$	$182.30\pm33.64$	$10802 \pm 29.01$	$244.95\pm6.09$	$33.87 \pm 0.76$	5523.96 ± 26.63	$89.27 \pm 3.74$	[g ii
f	6.56	1.17	0.52	1.29	11.23	2.40	2.32	Ъ
р	0.02	0.38	0.68	0.34	0.00	0.14	0.15	
Winter	$2512.21 \pm 1467.12$	$238.13 \pm 13.75$	$5768.01 \pm 435.14$	$139.95 \pm 7.22$	$120.48\pm12.22$	$2161.88 \pm 82.74$	$25.61 \pm 16.20$	90
Spring	$596.89 \pm 430.82$	$112.16\pm4.11$	$7771.98 \pm 166.26$	$160.79\pm0.78$	$54.80 \pm 43.44$	1863.27 ± 39.53	$74.84 \pm 1.64$	ien
Summer	$2620.86 \pm 493.47$	$193.03 \pm 13.32$	$16636.36 \pm 212.49$	$257.89 \pm 5.81$	$6.29 \pm 6.29$	3907.57 ± 59.67	$51.67 \pm 1.30$	š
Autumn	$6821.27 \pm 1190.21$	$102.74 \pm 5.86$	9364.29 ± 56.14	$102.03 \pm 1.46$	$239.03\pm47.89$	$2972.88 \pm 14.95$	$60.43 \pm 3.21$	5
f	6.90				9.30			UWC or Science
р	0.01				0.01			Þ
Winter	$3820.08 \pm 241.15$	$117.65 \pm 20.77$	$9444.31 \pm 245.32$	$133.78 \pm 6.40$	$277.42 \pm 3.07$	$1808.26 \pm 65.17$	$96.24 \pm 2.89$	
Spring	$3322.38 \pm 1278.02$	$90.89 \pm 14.45$	$9379.69 \pm 141.29$	$119.43 \pm 2.24$	$411.29\pm65.29$	$1704.63 \pm 11.46$	$92.67 \pm 0.15$	t
Summer	$3469.33 \pm 1414.86$	$111.39\pm4.78$	$14796.28 \pm 56.04$	$279.95\pm3.83$	$279.95\pm3.83$	$3383.84 \pm 14.57$	$187.84\pm1.41$	cest
Autumn	$3140.83 \pm 1943.72$	$40.22\pm20.21$	$7579.60 \pm 1068.96$	$59.11\pm29.56$	$257.83\pm16.21$	$1132.60 \pm 328.22$	$73.61\pm8.60$	Worcester
f	0.04	4.60			1.82			13
p	0.99	0.04			0.22			

Appendix 3:	Elemental	concentrations	for	Cyperus	longus	(mean	$\pm$	S.D)	(mg/kg)

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			Parameters for Typh	a capensis plan	t			
	E	lemental concen	tration for <i>Typha cap</i>	<i>ensis plant</i> (me	an ± S.D) (mg/kg)			
Season	Ca	Fe	K	Mg	Na	Zn	Location	
Winter	$2300.80 \pm 826.80$	$141.39 \pm 10.55$	17339.95 ± 318.36	$172.33 \pm 4.54$	3591.73 ± 85.77	$173.06 \pm 4.59$		
Spring	$2979.75 \pm 958.25$	$93.58 \pm 17.68$	$12058.33 \pm 916.41$	$167.63 \pm 5.67$	$3954 \pm 201.93$	$131.52 \pm 27.93$	*	
Summer	$2459.29 \pm 1014.38$	$74.56 \pm 7.65$	$10654.73 \pm 89.81$	$149.31 \pm 9.20$	$4333.15 \pm 177.03$	$94.81 \pm 20.47$	no	
Autumn	$1564.98 \pm 140.81$	$122.74 \pm 11.33$	$11256.88 \pm 514.90$	$118.78 \pm 9.92$	$3420.38 \pm 403.73$	$93.60 \pm 11.06$	Grabouw	
f	0.52	5.80		10.00	2.71	3.39	0	
p	0.68	0.02		0.00	0.12	0.07		
Winter	1390.07 ± 436.24	39.30 ± 18.60	25045.03 ± 3963.27	$165.08 \pm 5.46$	$5172.92 \pm 906.30$	$46.01 \pm 4.46$		
Spring	$6952.93 \pm 761.75$	$54.54 \pm 31.22$	$16836.61 \pm 382.77$	$158.29\pm2.18$	$6631.08 \pm 21.72$	$60.69 \pm 1.46$	斑	
Summer	$6942.53 \pm 1668.79$	$82.17 \pm 23.24$	$20279.29 \pm 1894.22$	$214.46\pm38.38$	$7409.84 \pm 399.32$	$110.45\pm28.47$	Kelderhoff	
Autumn	$5050.88 \pm 1283.10$	$90.11 \pm 16.78$	$16137.18 \pm 1390.83$	$184.74 \pm 23.69$	$5776.69 \pm 645.93$	$56.11 \pm 5.35$	elde	
f	5.28	1.05	3.10	1.23	2.74	3.84	м	
р	0.03	0.42	0.09	0.36	0.11	0.06		
Winter	$4831.88 \pm 715.38$	$95.10\pm54.74$	$12416.99 \pm 845.48$	$160.42 \pm 15.80$	$6571.22 \pm 300.93$	$56.76 \pm 4.53$		
Spring	$7246.22 \pm 1748.25$	$53.43 \pm 14.33$	$14267.32 \pm 156.96$	$141.50\pm371$	$7337.12 \pm 43.62$	$46.97 \pm 1.69$	ay	
Summer	$1161.08 \pm 206.59$	$137.35\pm13.59$	$11722.62 \pm 1137.18$	$136.25\pm8.66$	$5055.83 \pm 1141.19$	$89.43 \pm 24.61$	Pringle Bay	
Autumn	$788.73 \pm 423.17$	$98.63 \pm 25.94$	$13561.89 \pm 515.17$	$139.41\pm10.37$	$6127.44 \pm 525.23$	$56.21 \pm 6.32$	ing.	
f	10.07	1.16	2.26	1.07	2.17	2.07	Ъ	
р	0.00	0.38	0.16	0.42	0.17	0.18		
Winter	509.49 ± 509.49	$142.73\pm7.37$	$20126.12 \pm 441.84$	$86.91 \pm 0.69$	$3664.78 \pm 21.57$	$100.00\pm5.08$	ſĊ	
Spring	$753.77 \pm 497.64$	$80.54 \pm 13.95$	$16406.96 \pm 60.43$	$96.04 \pm 2.83$	$5039.10 \pm 60.80$	$94.97 \pm 1.68$	B	
Summer	$1782.98 \pm 433.82$	$92.01\pm8.30$	$15095.21 \pm 2154.66$	$79.19 \pm 2.32$	$3061.64 \pm 33.87$	$75.54 \pm 13.68$	Science or UWC	
Autumn	$4129.93 \pm 1030.25$	$83.56 \pm 10.15$	$12041.88 \pm 1194.73$	$89.33 \pm 7.53$	$3289.07 \pm 282.22$	$60.21 \pm 5.04$	106	
f	6.22	8.04	7.16	2.75		5.57	ciei	
р	0.02	0.01	0.01	0.11		0.02	S	
Winter	$2895.74 \pm 483.15$	$158.50\pm52.23$	$11997.86 \pm 1829.08$	$225.03\pm20.52$	$5964.40 \pm 1570.04$	$35.73 \pm 4.01$		
Spring	$1102.55 \pm 434.04$	$192.43\pm56.91$	$14215.75\pm 585.74$	$280.93\pm46.59$	$6228.92 \pm 2157.18$	$35.53\pm5.69$	er	
Summer	$3356.40 \pm 1166.54$	$268.17\pm13.22$	$10977.40 \pm 633.83$	$324.44\pm20.44$	$7290.26 \pm 2681.57$	$59.61 \pm 18.95$	Worcester	
Autumn	$2887.48 \pm 284.58$	$262.55 \pm 11.89$	$9815.53 \pm 1560.63$	$305.74\pm37.57$	$3280.21 \pm 2246.40$	$44.95\pm3.30$	lord	
f	2.13	1.84	2.14	1.69	0.60	1.23	ы	
р	0.18	0.22	0.17	0.25	0.63	0.36		

Appendix 4: Elemental concentrations for *Typha capensis* (mean  $\pm$  S.D) (mg/kg)

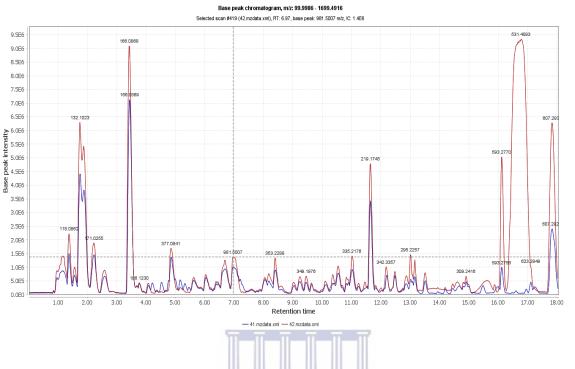
		Centella asiatica		Cyperus longus			Typha capensis		
	Season	pН	Conductivity (µs/cm)	pН	Conductivity(µs/cm)	pН	Conductivity (µs/cm)		
R	Winter			7.52	1770.02	5.58	401.38		
no	Spring			7.39	201.37	6.75	322.42		
Grabouw	Summer			7.29	289.52	5.81	220.1		
0	Autumn			5.87	129.96	5.27	311.23		
Ħ	Winter			6.76	322.42	7.09	917.91		
Kelderhoff	Spring			5.83	220.1	7.41	888.3		
elde	Summer			8.06	586.61	7.74	1398.25		
м	Autumn			7.16	518.5	7.37	376.25		
rth	Winter	8.07**	586.61						
MO	Spring								
Kenilworth	Summer	7.16**	518.83						
М	Autumn								
b ay	Winter			7.08	839.79	6.75	403.34		
Pringle B	Spring			6.97	334.11	7.03	215.52		
ng 11	Summer			6.75	205.63	5.68	984.27		
	Autumn			7.52	776.58	5.71	915.04		
or U	Winter			5.27	311.23	7.15	210.7		
000	Spring			7.17	690.9	7.64	236.89		
Science	Summer			7.5	210.7	5.51	571.6		
Sci	Autumn			7.52	704.06	5.87	120.7		
er	Winter			6.21	171.57	6.21	171.57		
Worcester	Spring			6.62	135.45	7.64	486.92		
/or	Summer			6.14	177.29	7.67	2401.98		
12	Autumn			5.44	620.06	8.14	1562.75		

# Appendix 5: Mean pH and conductivity

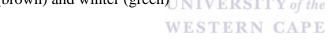
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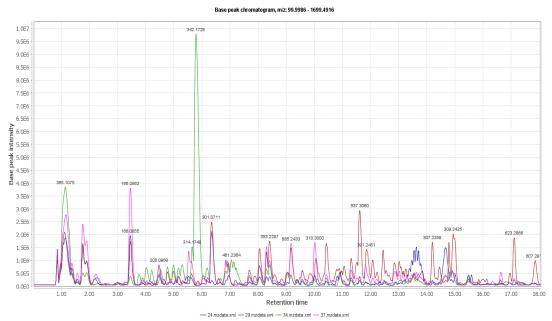
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**Appendix 6:** Chromatograms of *Centella asiatica* metabolites in winter (blue) summer (red). Pringle Bay

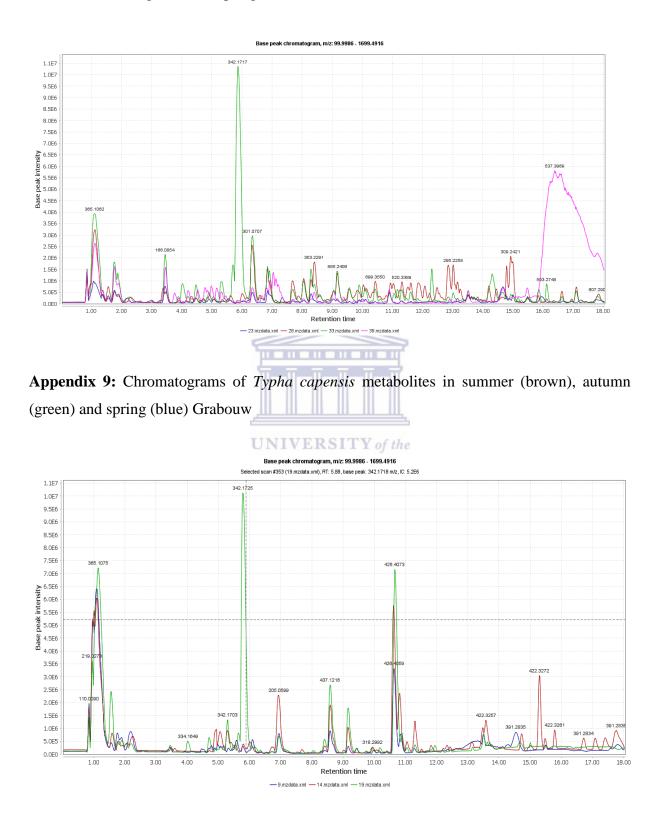


Appendix 7: Chromatograms of *Cyperus longus* metabolites in spring (blue) summer (pink) autumn (brown) and winter (green)

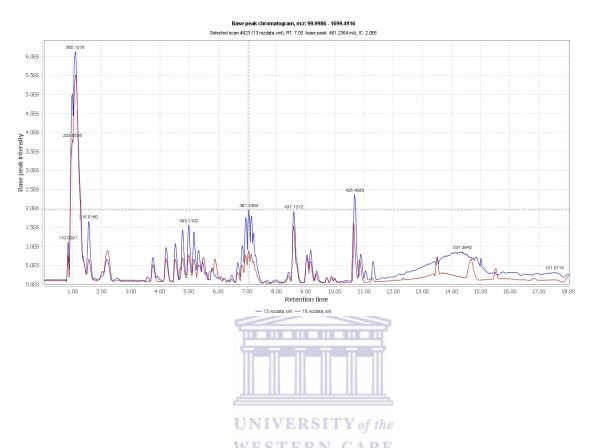




**Appendix 8:** Chromatograms of *Cyperus longus* metabolites in winter (purple), summer (brown), autumn (green) and spring (blue)



**Appendix 10:** Chromatograms of *Typha capensis* metabolites in summer (blue) and autumn (brown) Pringle Bay



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